# MANGANESE UTILIZATION BY RUMINANTS AND POULTRY

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TABLE OF CONTENTS


| | | Page |
|---|---|---|
| ACKNOWLEDGEMENTS | | ii |
| LIST OF TABLES | | vi |
| LIST OF APPENDIX TABLES | | viii |
| LIST OF FIGURES | | ix |
| ABSTRACT | | x |
| CHAPTER I | INTRODUCTION | 1 |
| II | LITERATURE REVIEW | 3 |
| | Functions of Manganese | 4 |
| | Manganese Deficiency | 6 |
| | Rats and mice | 6 |
| | Poultry | 7 |
| | Swine | 9 |
| | Ruminants | 10 |
| | Factors Affecting Manganese Requirement | 11 |
| | Manganese Absorption and Excretion | 13 |
| | Blood Clearance and Tissue Deposition of Manganese | 16 |
| | Manganese Toxicity | 17 |
| III | EXPERIMENT 1. INFLUENCE OF DIETARY MANGANESE LEVEL ON THE METABOLISM $^{54}$Mn, STABLE MANGANESE AND OTHER MINERAL ELEMENTS | 22 |
| | Experimental Procedure | 23 |
| | Results | 26 |
| | Absorption, Excretion, Blood Clearance and Deposition of $^{54}$Mn | 26 |
| | Absorption, Excretion and Blood Levels of Stable Manganese | 36 |
| | Apparent Digestion and Tissue Deposition of Dietary Constituents | 39 |
| | Discussion | 45 |
| | Summary | 48 |

CHAPTER | Page

IV EXPERIMENTS 2 AND 3. BIOLOGICAL ASSAY OF INORGANIC MANGANESE FOR CHICKS ..... 50

Experimental Procedure ..... 51
Results ..... 55
Discussion ..... 64
Summary ..... 65

V EXPERIMENTS 4 AND 5. BIOLOGICAL AVAILABILITY OF SEVERAL INORGANIC FORMS OF MANGANESE TO CHICKS ..... 67

Experimental Procedure ..... 67
Results ..... 69
Discussion ..... 84
Summary ..... 88

VI SUMMARY AND CONCLUSIONS ..... 90

Absorption, Blood Clearance, Tissue Deposition and Excretion of Manganese and Other Elements in Sheep ..... 90
Biological Assay for Manganese Availability in the Chick ..... 92

APPENDIX ..... 94

BIBLIOGRAPHY ..... 102

BIOGRAPHICAL SKETCH ..... 109

LIST OF TABLES


| TABLE | | Page |
|---|---|---|
| 1 | COMPOSITION OF BASAL DIET | 24 |
| 2 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON ACCUMULATED PERCENT OF THE $^{54}Mn$ DOSE EXCRETED IN THE FECES | 27 |
| 3 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON ACCUMULATED PERCENT OF THE $^{54}Mn$ DOSE EXCRETED IN THE URINE | 30 |
| 4 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON TISSUE RETENTION OF RADIOACTIVITY | 34 |
| 5 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON THE SPECIFIC ACTIVITY IN TISSUE | 35 |
| 6 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON DAILY ABSORPTION AND EXCRETION OF STABLE MANGANESE | 37 |
| 7 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON HEMATOCRIT AND PLASMA MANGANESE VALUES | 38 |
| 8 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON DIGESTIBILITY OF ORGANIC MATTER AND DRY MATTER AND APPARENT ASH ABSORPTION | 40 |
| 9 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON PERCENT APPARENT ABSORPTION OF MINERAL ELEMENTS BY SHEEP | 41 |
| 10 | MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON TISSUE MINERAL COMPOSITION OF SHEEP | 42 |

| TABLE | | Page |
|---|---|---|
| 11 | CHEMICAL COMPOSITION OF MANGANESE SUPPLEMENTS, THEIR SOLUBILITY IN VARIOUS SOLVENTS AND X-RAY PATTERNS | 52 |
| 12 | COMPOSITION OF BASAL DIET | 53 |
| 13 | BODY WEIGHTS OF 28-DAY OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE (EXPERIMENTS 2 AND 3) | 56 |
| 14 | LEG ABNORMALITY SCORES OF 28-DAY OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE (EXPERIMENTS 2 AND 3) | 58 |
| 15 | BONE ASH OF 28-DAY OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE | 59 |
| 16 | TIBIA BREAKING STRENGTH OF 28-DAY OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE | 60 |
| 17 | BONE (TIBIA PLUS FEMUR) MANGANESE LEVELS OF 28-DAY OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE | 61 |
| 18 | MANGANESE CONTENT, SOLUBILITY AND X-RAY PATTERNS OF MANGANESE SUPPLEMENTS | 68 |
| 19 | BODY WEIGHTS OF 28-DAY OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE | 71 |
| 20 | LEG ABNORMALITY SCORES OF 28-DAY OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE | 72 |
| 21 | PERCENTAGE BONE ASH IN TIBIA AND FEMUR FROM 28-DAY OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE | 77 |
| 22 | BONE (TIBIA PLUS FEMUR) MANGANESE LEVELS OF 28-DAY OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE | 78 |
| 23 | BONE (TIBIA PLUS FEMUR) MANGANESE LEVEL OF 28-DAY OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE | 80 |
| 24 | RELATIVE AVAILABILITY OF MANGANESE TEST SOURCES AT A SUPPLEMENTAL LEVEL OF 10 PPM | 87 |

LIST OF APPENDIX TABLES


| TABLE | | Page |
|---|---|---|
| 25 | LIVER LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$..... | 95 |
| 26 | KIDNEY LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$..... | 96 |
| 27 | HEART LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$..... | 97 |
| 28 | SPLEEN LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$..... | 98 |
| 29 | MUSCLE LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$..... | 99 |
| 30 | BONE LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$..... | 100 |
| 31 | BRAIN LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$..... | 101 |

LIST OF FIGURES


| FIGURE | | Page |
|---|---|---|
| 1 | Accumulated Percent of $^{54}Mn$ Dose Excreted in the Feces | 28 |
| 2 | Accumulated Percent of $^{54}Mn$ Dose Excreted in the Urine | 31 |
| 3 | Plasma $^{54}Mn$ Levels of Lambs Fed Different Levels of Dietary Manganese After IV $^{54}Mn$ Dose | 32 |
| 4 | Relation of Bone (tibia plus femur) Manganese Levels to Manganese Intake in the Form of Manganese Sulfate | 63 |
| 5 | Representative Tibiae from 28-Day Old Chicks Raised on Varying Levels of Dietary Manganese in Experiment 5 | 74 |
| 6 | Representative Tibiae from 28-Day Old Chicks Raised on a Diet Containing 10 ppm Supplemental Manganese Supplied from Various Sources in Experiment 5. Manganese Sources Represented from Left to Right are: No.'s 2, 4, 6, 8, 12 and 13. | 75 |
| 7 | Relation of Bone (tibia plus femur) Manganese Levels to Manganese Intake in the Form of Manganese Sulfate (Experiment 4) | 82 |
| 8 | Relation of Bone (tibia plus femur) Manganese Levels to Manganese Intake in the Form of Manganese Sulfate (Experiment 5) | 83 |

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# MANGANESE UTILIZATION BY RUMINANTS AND POULTRY

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Studies were conducted to obtain information on certain nutritional aspects of manganese in sheep and poultry. The data obtained involved the effects of different levels and sources of dietary manganese on the utilization of manganese and other mineral elements within the animal body. In Experiment 1, lambs receiving a basal diet, containing 30 ppm manganese, or the basal diet plus 4,000 ppm supplemental manganese were dosed either orally or intravenously with $^{54}Mn$. Limited intestinal absorption of manganese was demonstrated by low values recorded for apparent absorption and net retention of stable manganese. In addition, plasma and tissue uptake of $^{54}Mn$ by those animals receiving oral doses of the radioisotope was very low. Excretion of manganese from the body was almost exclusively by way of the feces regardless of dietary manganese level or pathway of radioisotope administration. The tissues, listed in decreasing order of enrichment with radioactive manganese, were as follows: kidney, liver, spleen, brain, heart, bone and muscle. The greatest tissue retention of radioactive manganese was demonstrated by the treatment group fed the basal diet and given the radioisotope intravenously. Lambs fed the manganese-supplemented diet had higher levels

of stable manganese in the tissues and plasma than those fed the unsupplemented basal diet. The high manganese diet also resulted in decreased intestinal absorption of iron and phosphorus and decreased liver concentrations of iron and zinc.

Experiments 2 and 3 were conducted to develop a suitable biological assay for manganese availability with chicks. Reagent-grade manganese sulfate was chosen as the reference standard and added to a semi-purified basal diet at levels of 10, 20 and 30 ppm manganese. The other test materials, two commercial feed-grade manganese oxides, were supplemented at levels of 10 and 20 ppm manganese to the same diet. Ten ppm manganese from the sulfate or one of the oxide sources were adequate for normal growth and bone ash and prevented the occurrence of perosis. However, 10 ppm manganese from the other test source were inadequate for normal responses. Bone manganese levels were found to be more directly related to dietary manganese levels than the other responses measured. Thus, it was suggested that bone manganese concentration may be sufficiently sensitive to serve as a response criterion in a manganese availability assay when manganese sulfate is used as the standard source.

Experiments 4 and 5 were duplicate trials designed to determine the availability of manganese from several inorganic sources using the assay method suggested in Experiments 2 and 3. The reference standard, manganese sulfate, was supplemented to the basal semi-purified diet at levels of 10, 20, 30, 60 and 120 ppm manganese. Six other inorganic manganese sources were tested at a supplemental manganese level of 10 ppm. Body weight and percentage bone ash were not sensitive enough as response criteria to detect differences in biological availability among sources of manganese. The test sources differed in their effectiveness in

preventing perosis. This indicates that incidence of leg abnormalities is a useful response criterion for manganese availability at dietary levels of 10-15 ppm manganese. Above this level, however, abnormalities appear to be prevented entirely.

A highly significant correlation existed between bone manganese concentration and dietary levels of the element. Bone manganese increased linearly with increasing dietary manganese, at least up to a dietary level of 35 ppm total manganese. Thus, it was concluded that tests for biological availability of manganese should be conducted at dietary levels below 35 ppm in the area of maximum response. In these experiments, differences in availability of manganese from test sources were found when bone manganese levels were expressed as percentages relative to the response obtained with the standard, manganese sulfate, when supplied at the same dietary manganese level.

# CHAPTER I

## INTRODUCTION

Mineral elements constitute only 4 to 6 percent of the vertebrate body. More specifically, manganese makes up about 0.0003 percent - a very small but important amount. Manganese is necessary for growth, bone formation, and reproduction. However, attempts to delinate the specific biochemical role or roles of the element have failed.

Manganese has been known to be a constituent of plant and animal tissue for more than 50 years. It was first demonstrated to be an essential element for animals in 1931 (Kemmerer, Elvehjem and Hart). Nutritional research with manganese gained practical significance in 1936 (Wilgus, Norris and Heuser) and 1937 (Lyons and Insko) when it was discovered that two commonly occurring diseases in poultry, perosis and nutritional chondrodystrophy, could be prevented by manganese supplementation.

The manganese content of soils and plants has been demonstrated to be quite variable. Nutritional disabilities attributed to manganese deficiency have been reported with grazing cattle under field conditions in England, the Netherlands (Underwood, 1966; and Bourne, 1967) and the United States (Dyer, 1961). In other areas, where the manganese content of soils is relatively high, grazing cattle have shown signs of

antagonistic relationships between manganese and other elements (Gallup et al., 1952).

Numerous research studies have been conducted concerning the dietary requirements for manganese. The requirement has been found to be quite variable, being affected by such factors as the species and breed of the animal being considered, the age of the animal, the chemical form of the manganese being offered, and the level of other elements in the diet which have been shown to be antagonistic to manganese utilization.

The experiments reported herein were conducted to study the effects of level and source of dietary manganese on metabolism and animal performance in ruminants and poultry. In Experiment 1 sheep were fed either high or low levels of dietary manganese and dosed either orally or intravenously with $^{54}Mn$, a radioisotope of manganese. Measurements of absorption, excretion, blood clearance and tissue deposition of both the radioactive and stable isotope of manganese were made. Experiments 2, 3, 4 and 5 were conducted with chicks in an effort to develop a suitable biological assay for manganese availability. Many commercial inorganic sources of manganese exist; however, their relative values as dietary supplements are unknown. These experiments used growth, leg development, percent bone ash and manganese content of bones as criteria for availability of the element from several inorganic sources.

## CHAPTER II

## LITERATURE REVIEW

The biology of manganese suffers from the lack of unifying principles. A vast accumulation of information and experience exists concerning manganese metabolism, but no precise function can be ascribed to the metal *in vivo*. Since Bertrand (1913) demonstrated the presence of manganese in plant and animal tissues it has been found to be essential for normal growth, skeletal development, reproductive performance and function of the central nervous system. However, its exact biochemical function or functions remain unknown.

The absence of other unknown but essential nutrients from experimental diets and the trace amounts of manganese required by most animal species caused early attempts to demonstrate the essentiality of manganese to be unsuccessful. It was not until 1931 that two groups of workers independently discovered that manganese is necessary for growth and fertility in mice and rats (Kemmerer, Elvehjem and Hart, 1931; and Orent and McCollum, 1931).

Manganese first became of practical significance to the nutritionist in 1936 and 1937 when two poultry diseases, perosis or "slipped tendon" (Wilgus, Norris and Heuser, 1936 and 1937) and nutritional chondrodystrophy (Lyons and Insko, 1937), were found to be caused by inadequate intakes of manganese from certain practical diets and could

be prevented by supplementation of the element to these diets. These findings led to extensive investigations of dietary requirements and tissue concentrations of manganese in different animal species.

## Functions of Manganese

The specific function or functions of manganese, as stated earlier, are still unknown. The element, however, has been suggested as functional in several processes within the body. Since the most pronounced physical manifestations of manganese deficiency are bone abnormalities, manganese has been implicated in the calcification or formation of bone. Wiese et al. (1939) reported that blood and bone phosphatase activity in chicks with manganese deficiency is lower than normal and suggested that manganese might serve as an activator for this enzyme. This finding was duplicated in swine (Liebholz, Speer and Hays, 1961), cattle (Rojas, Dyer and Cassatt, 1965) and sheep (Lassiter and Morton, 1968). However, manganese deficiency in rats has been shown to have no effect on phosphatase activity by two groups of researchers (Wachtel, Elvehjem and Hart, 1943; and Hurley, Everson and Geiger, 1959).

Leach and Muenster (1962) and Leach (1968) found lesions in the epiphyseal plate before external symptoms of bone weakness appeared in chicks fed a manganese-deficient diet. They found that osseous tissue from manganese-deficient chicks had decreased levels of chondroitin sulfate, and the epiphyseal plate showed the greatest reduction in this matrix constituent. Thus, they suggested that manganese is required for proper formation of the organic matrix of cartilage and bone. Savage (1968) also supported this view in a review of manganese nutrition in poultry species stating that manganese probably functions in the

maintenance of proper amounts of mucopolysaccharides in epiphyseal cartilage and bone.

It has been suggested also that manganese serves as activator for the enzyme arginase by Boyer, Shaw and Phillips (1942) and Wachtel, Elvehjem and Hart (1943) since a manganese deficiency in rats resulted in a decreased activity of this enzyme.

Cotzias (1958) postulated that since birds require more manganese than other species and have a higher body temperature with more oxygen consumption, maybe manganese is involved in some oxidation-reduction processes. In 1954, Lindberg and Ernster reported that the catalytic units of the respiratory chain capable of generating energy-rich phosphate bonds occur in two forms: (1) a non-phosphorylative form, or an enzyme-coenzyme complex; or (2) a phosphorylative form containing adenosine-triphosphate as a cofactor and linked to the non-phosphorylative form by means of manganese. If this is the function of manganese, then it would necessarily appear in relatively great concentrations within the mitochondria where the respiratory chain operates. This indeed was confirmed by Maynard and Cotzias (1955) when they demonstrated that tissues with the highest uptake (liver and kidney) of intraperitoneally injected radioactive manganese are also rich in mitochondria. About one-half of the manganese activity found in these tissues was found to be located in the mitochondria. Thus, it is possible that the concentration of manganese in mitochondria is a reflection of its role as a respiratory cofactor.

## Manganese Deficiency

### Rats and Mice

Orent and McCollum (1931) demonstrated that testicular degeneration occurred in rats raised on a manganese deficient diet. In the same year, Kemmerer, Elvehjem and Hart (1931) found that female mice raised on an all-milk diet grew poorly and failed to ovulate normally upon reaching maturity. When manganese was supplemented in the diet, growth was stimulated and ovulation was normal.

It was reported by Skinner, Van Donk and Steenbock (1932) that female rats raised on an all-milk diet fortified with iron and copper were slow in attaining sexual maturity. However, when manganese was added to the same diet at a level of about 10 ppm, first estrous was exhibited at the normal age. In further studies with rats raised on diets either low or practically devoid of manganese, Boyer, Shaw and Phillips (1942) found that in manganese-deficient females, estrous cycles were absent or irregular with a delay in the opening of the vaginal orifice. The same authors showed that testicular degeneration and sterility resulted in male rats grown on low-manganese diets. They also recorded a reduced concentration of liver arginase in manganese-deficient rats.

Watchel, Elvehjem and Hart (1943) fed a diet supplying five micrograms of manganese per day to growing rats. These animals exhibited an impaired growth rate, which was more pronounced with higher calcium to phosphorus ratios. They also had poor bone formation, having 5 percent less bone ash than those animals receiving a control diet. In addition, deficient rats were slightly anemic and had a reduced liver

arginase activity. More recently, Hurley et al. (1961) showed that feeding a milk diet containing 0.13 ppm manganese to growing rats resulted in reduced total body length as well as reduced length of long bones. Tibias were greatly thickened and distorted in shape as compared to tibias from identical rats receiving the basal milk diet supplemented with 6 ppm manganese.

Poultry

Perosis, as described by Gallup and Norris (1939a), is a deficiency disease affecting the development of bone. The characteristic symptoms are enlargement of the tibio-metatarsal joint, bending of the distal end of the tibia and proximal end of the metatarsus and displacement of the achilles tendon. Locomotion is impaired, and in severe cases the animal is unable to stand. The outward manifestations of injury give rise to the descriptive name "slipped tendon".

Titus and Ginn (1931) and Titus (1932) first proposed the name perosis and stated that rice bran contained a factor that prevents the disease. Heller and Penquite in 1936 and 1937 reported a protective factor in a water-soluble extract of rice bran. In that same year, Sherwood and Fraps (1936) found that the ash of wheat gray shorts was protective against perosis and thus concluded that the factor was inorganic in nature.

Wilgus, Norris and Heuser (1936 and 1937) were first to link perosis with a manganese deficiency. They stated that manganese and other trace elements were essential for the prevention of perosis in chickens. Their research showed that the perosis-preventing properties of cereals are directly related to their manganese content. They fed

a diet containing 10 ppm manganese which resulted in a high incidence of perosis. However, when 10 to 15 ppm manganese were added to this diet, growth was stimulated and perosis was prevented entirely. A supplement of zinc and aluminum seemed essential but was less effective in preventing the disorder. They found that iron at high levels seemed to increase the manganese requirement, a result which was later substantiated. They also found that steamed bone meal as well as pure calcium phosphate aggravated the occurrence of perosis or increased the manganese requirement. Insko, Lyons and Martin in 1938 also reported that manganese is protective against perosis, but contrary to the results of Wilgus they reported that 30 ppm each of aluminum and zinc in the ration did not protect but actually increased the incidence of perosis.

Caskey and Norris (1938) found that a diet containing 15 ppm manganese, 1.0 percent calcium and 0.5 percent phosphorus was more effective in preventing perosis than the same basal diet containing 140 ppm manganese, 3.0 percent calcium and 1.5 percent phosphorus. They postulated that high calcium and phosphorus levels block the absorption of manganese from the intestinal tract as will be discussed later.

Lyons and Insko (1937) demonstrated that another nutritional disease of poultry, termed nutritional chondrodystrophy, could be prevented by manganese supplementation. This is a disease of the unborn fetus which results in gross skeletal malformations and embryo mortality. These authors found that supplementation of 40 ppm manganese to the diet of hens or injection of 0.03 milligrams of the element into the egg would prevent the disease entirely. Gallup and Norris (1939b) reported that a layer diet containing 13 ppm manganese resulted in low

egg production and a high incidence of embryo mortality. They also found that when chicks were hatched from these eggs, they had low reserves of the element and were susceptible to perosis. The manganese content of the egg was found to be directly related to the dietary manganese level of the hen. Hill and Mathers (1968) reported that the dry matter of eggs from pullets receiving 6.5 or 50 ppm dietary manganese contained 0.66 and 1.50 ppm of the element, respectively. In 1968, Savage demonstrated that manganese is required at dietary levels of from 54 to 108 ppm in turkeys for normal egg production and hatchability.

Swine

Miller et al. (1940) reported that corn-soy type diets containing 11-14 ppm manganese caused lameness in 50 percent of the pigs receiving such diets. They found that manganese sulfate added at levels of 50 to 60 ppm manganese prevented the lameness but were ineffective in curing stiffness after it had developed. Johnson (1943 and 1944) demonstrated satisfactory growth of pigs receiving natural feedstuffs containing 7-10 ppm manganese from weaning to market weight. Reproduction was normal through two generations in pigs raised on these feedstuffs. However, when pigs were raised on a diet containing 0.5 ppm manganese they failed to reproduce normally. Reproduction was satisfactory when the manganese level of this diet was raised to 6 ppm. Grummer et al. (1950) showed that growth of pigs confined to a concrete feeding floor and receiving a corn-soy ration containing 12 ppm manganese could be improved by the addition of 40 ppm manganese to the basal diet. In contrast, Liebholtz, Speer and Hays (1961) found no growth depression or adverse feed utilization by pigs fed a diet

containing only 0.35 ppm of manganese. However, they found a decrease in bone manganese and alkaline phosphatase activity.

Plumlee et al. (1956) also found no difference in rate of gain or feed efficiency over the growth period of pigs fed a semi-purified diet containing from 0.5 to 40 ppm manganese. Boars raised on a dietary level of 3.3 ppm manganese grew normally and showed normal spermatogenesis. However, gilts fed through growth, gestation and lactation on a diet containing 0.5 ppm manganese exhibited reduced skeletal growth and weakness, irregular estrus cycles, fetal resorption, low milk production and decreased tissue levels of manganese.

Ruminants

Bentley and Phillips (1951), in experiments with the effects of low dietary manganese on dairy cattle, reported that 10 ppm manganese in the diet were adequate for growth of heifers since higher levels did not stimulate faster growth. However, the heifers receiving only 10 ppm manganese were slower to exhibit their first estrous, were slower to conceive upon breeding, and gave birth to a higher percentage of calves with leg deformities than did control heifers raised on diets containing 30 ppm manganese. The cattle were slaughtered after three lactations with post-mortem examination and analysis revealing no differences in tissue concentration of manganese except in the ovaries, but revealing abnormal structural changes in the liver of those animals receiving the lower manganese level in the diet. They concluded that 20 ppm manganese in the diet seemed adequate for growth and reproduction under normal conditions. However, Dyer, Cassatt and Rao (1964) reported that 21 ppm manganese were inadequate for gestating beef heifers, resulting

in leg deformities in the newborn and reduced liver and bone concentrations of manganese in the dam. They found 61 ppm to be the minimum level of manganese which resulted in normal calves with normal tissue concentrations of the element. Dyer and Rojas (1965) reported that 45 ppm seemed adequate for reproduction. In continued work with manganese deficiency in cattle, Rojas, Dyer and Cassatt (1965) found that 16 ppm or less of manganese were inadequate for reproduction resulting in calves with neonatal deformities with reduced breaking strength and length of the humerus, reduced serum alkaline phosphatase activity, and lower manganese levels of bone, liver, and gonads. They suggested that about 20 ppm of manganese would be adequate for reproduction, but that ingestion of compounds antagonistic to manganese utilization may increase the dietary requirement of the element.

In recent work by Lassiter and Morton (1968), effects of low manganese diets fed to lambs were studied. Four sets of twin ewe lambs were used, one from each set receiving 0.8 ppm of manganese in a purified diet, the other receiving 29.9 ppm in the same diet. After 16 weeks those receiving low dietary manganese exhibited weak joints, a decreased feed intake, and a reluctance to move. The lambs were slaughtered at 22 weeks with post-mortem examination showing that those fed the low manganese diet had shorter tibias with less breaking strength and a reduced ash content.

## Factors Affecting Manganese Requirement

The requirements for manganese are quite variable and are affected by the criteria of adequacy employed, the chemical form, the nature of the diet, and the breed and species of the animal under consideration.

The amount of manganese required for reproduction and other stress conditions is higher than that for growth and maintenance. Bentley and Phillips (1951) found, and their results were substantiated by Garrett (1964), that 9 to 10 ppm of manganese were adequate for growth of calves. However, they found this amount to be entirely inadequate for normal sexual maturity to be attained. Therefore, a level of dietary manganese may be high enough for growth, but inadequate for reproduction.

Differences in biological availability of manganese from different sources or chemical forms can also alter the animal's requirement for the element. Schaible, Bandemer and Dividson (1938) reported that practically all chemical forms of manganese are of equal value to the animal except for a few carbonate and silicate ores. They stated that animals receiving diets high in corn require supplemental manganese since corn, as is true for many small grains, has a very low manganese content. They reported that manganese content of grain and forage plants varies according to cutting time, stage of maturity, fertilization and soil reaction. Soil reaction seemed to be the most important factor with manganese being much higher in plants grown on acid soils.

Gallup and Norris (1939a), working with manganese requirements for chicks, found that manganese chloride, sulfate, carbonate, and oxide were of equal value in preventing perosis when supplemented at a level of 50 ppm manganese to a basal diet containing 10 ppm of the element naturally. Combs (1951) also stated that the chemical forms: manganese sulfate, manganese chloride, manganese carbonate, manganese dioxide, potassium permanganate, and the manganese ores: manganite, pyrolusite, hausmannite and hematite serve as satisfactory sources of manganese for the chick. He found that a manganese oxide ore was 85 percent as

effective as manganese sulfate in preventing perosis when supplemented at dietary levels of 15 or 25 ppm manganese but was of equal value when added at levels of 35 and 55 ppm.

Hennig et al. (1967) more recently compared the manganese uptake of broilers from various radioactive compounds including the sulfate, chloride and oxide forms of manganese. They found that radioactive manganese as manganous chloride was incorporated into the body to a significantly larger extent than that from the other compounds tested.

The requirement for manganese is quite variable among species as discussed earlier, with poultry species having a higher dietary requirement than most others. Differences have also been reported in the manganese requirements of breeds and strains within species as demonstrated in chickens by Gallup and Norris (1939a). In one strain of the New Hampshire breed, 50 ppm of supplemental manganese added to a basal diet containing 10 ppm of manganese reduced the occurrence of perosis from 80 percent to 4 percent while in another strain which seemed more susceptible to the disease, the same level of supplemental manganese only reduced the level of perosis to 18 percent. They found also that 30 ppm of supplemental manganese were as protective against perosis in the White Leghorn breed as were 50 ppm in the New Hampshire breed.

## Manganese Absorption and Excretion

Dietary requirements for manganese seem quite high in comparison to the trace concentrations of the element within the body. This is due to poor absorption of manganese from the gut and the antagonistic relationship which exists between manganese and other elements and compounds present in the diet or intestinal tract.

It was stated by von Oettingen (1935) that dietary manganese is slowly and incompletely absorbed from the intestinal tract, varying as to the acidity of the intestinal environment and the solubility of manganese compounds. He said further that manganese is excreted primarily in the colon and bile and only moderately in the urine. Skinner, Peterson and Steenbock (1931) reported that adult rats excreted from 80 to 99 percent of their dietary manganese in the feces depending on the level of manganese in the diet; more is excreted when an excess is present.

Greenburg, Copp and Cuthbertson (1943) found about 30 percent of an intraperitoneal injection of a radioactive manganese dose appearing in the bile within 48 hours. They stated that 50 to 75 percent of the injected dose appearing in the feces was carried by the bile. According to Cotzias (1958), manganese is absorbed in the rat at a level of 3 to 4 percent with excretion almost exclusively in the feces and significant excretion in urine only when abundant chelates are present. He also reported that manganese excreted into the bile or gut is partially reabsorbed. In a later paper Cotzias (1960) stated that excretion of manganese in bile is directly related to dietary intake.

Koshida, Kato and Hara (1963) and Kato (1963) presented evidence of manganese excretion through the intestinal epithelium. After the injection of a radioactive isotope of manganese they found conspicuous radioactivity in the intestinal epithelium and also in the intestinal mucus cohering to free surface. Papavasiliou, Miller and Cotzias (1966) reported that bile duct obstruction only diminished excretion of a radioactive dose of manganese. They stated that bile formation constitutes the main regulation of manganese excretion but, if overloaded, other excretion routes participate.

Brown and McCracken (1965) conducted a manganese balance trial with chickens using the isotope dilution technique. They found that when feeding a diet containing 45 ppm manganese to laying pullets, the apparent absorption of manganese was about 30 percent. Manganese retention was 1.6 milligrams per day which is high relative to the birds' requirement. Lassiter (1966) and Lassiter and White (1966) published results of manganese balance trials with sheep revealing that net absorption of the element varies between 8 and 19 percent, depending on the level of mineral supplementation in the diet used. They also reported that there was no detectable excretion of manganese in the urine.

Wilgus, Norris and Heuser (1936) found that the perosis-preventing properties of a diet were related to its manganese content as has been stated earlier. They also reported that excess calcium and phosphorus aggravated the condition. In 1939, Wilgus and Patton reported that excess calcium was essential for the stimulation of perosis. They found that calcium phosphate in the gut precipitated manganese ions from solution, and that feeding calcium phosphate as steamed bone meal decreased diffusible manganese in intestinal contents, thereby decreasing absorption and increasing the dietary manganese requirement. They also discovered that ferric hydroxide or ferric citrate had a similar effect on manganese if sodium chloride were present. Bourne (1967) stated that work with cattle in England also indicates that high levels of calcium and phosphorus in the diet increase the dietary manganese requirement by interfering with absorption of the element from the intestinal tract.

Blood Clearance and Tissue Deposition of Manganese

Borg and Cotzias (1958a and 1958b) and Cotzias (1963) have reported blood clearance values for rats and humans after intravenous injections of the radioisotopes of manganese, $^{54}Mn$ and $^{56}Mn$. They recorded a rapid disappearance of the element from the plasma. Within 10 minutes less than 10 percent of the dose remained in the plasma, and after 70 minutes radioactivity was only barely detectable in the plasma. Activity began to reappear in circulating red blood cells after 36 to 48 hours. They stated that most or all endogenous manganese is available for interchange within an hour and concluded that manganese must exist in the body in dissociable chelates or other relatively labile intracellular combinations.

It has been reported in swine (Plumlee et al., 1956), rats (Cotzias, 1963), and chickens (Settle et al., 1969) that after administration of radioactive isotopes of manganese, $^{54}Mn$ and $^{56}Mn$, the relative enrichment of the tissues with radioactivity listed in decreasing order was as follows: liver, kidney, spleen, heart, bone, muscle and brain. Kato (1963) using mice, and Rojas, Dyer and Cassatt (1966), using rats, found similar tissue responses to injection of $^{52}Mn$ and $^{56}Mn$ with one exception. They found that the kidney was more greatly enriched with radioactivity than the liver. The above researchers as well as Mathers and Hill (1967) found an inverse relationship between the dietary manganese level and the percent of the radioactive manganese dose retained by the various tissues. In contrast, Britton and Cotzias (1966) reported that the tissue concentration of $^{54}Mn$, following an injection, was directly related to the level of stable dietary manganese.

Thacker, Alderman and Bratton (1956) and Underwood (1962) have listed normal plasma levels of stable manganese to lie between 0.05 and 0.20 ppm. Results of tissue analyses in rats (Skinner, Peterson and Steenbock, 1931), swine (Johnson, 1943; and Svajgr, Peo and Vipperman, 1969), chickens (Mathers and Hill, 1968) and cattle (Bentley and Phillips, 1951; and Rojas, Dyer and Cassatt, 1965) are very similar indicating that manganese concentration of tissue varies from about 0.5 to 12.0 ppm. Tissues listed in decreasing order with respect to manganese concentration were: liver, kidney, pancreas, heart, bone and muscle. These authors also state that tissue concentrations of manganese are directly related to the dietary level of the element.

## Manganese Toxicity

The antagonistic relationship which exists between manganese and other elements and the wide variation in the level of manganese in common feedstuffs has stimulated the investigation of effects of high levels of dietary manganese. Becker and McCollum (1938) fed rats a diet containing manganese as manganese chloride at levels of 1,800, 3,600, 9,000, 18,000 and 36,000 ppm. Reproduction was normal at all levels of intake and growth was normal except at the 36,000 ppm level, at which level some growth depression was recorded. In 1942, Chornock, Guerrant and Dietcher reported that high dietary levels of manganese adversely affected growth of rats. They found that when manganese constituted 1.73 percent of the diet that calcium and phosphorus excretion in the feces was increased. There was a more pronounced interference with phosphorus retention than with calcium and an increase in dietary phosphorus improved its retention.

Gallup et al. (1952) investigated a condition in southeastern Oklahoma where cattle grazing a native grass in summer and fed native grass hay plus a protein supplement in the winter developed phosphorus deficiency unless phosphorus was supplemented. Cattle in north-central Oklahoma received the same or similar treatment but no phosphorus supplement was required. Analysis showed that the grasses in southeast Oklahoma had slightly less phosphorus but five to ten times as much manganese. They then conducted a mineral balance study in which the diet supplied 7.7 and 14.5 grams of calcium and phosphorus daily. Manganese was fed at graded levels of 0, 250, 1,000 and 2,000 ppm as manganous sulfate. It was found that phosphorus excretion in the feces was increased at all levels of manganese supplementation greater than zero. Calcium excretion was increased at 1,000 and 2,000 ppm manganese and calcium and phosphorus balances were positive as long as the manganese content of the diet was 500 ppm or less.

Working with lambs, Hartman, Matrone and Wise (1955) investigated manganese--iron interrelationships. After depletion of their body iron stores, lambs were fed a milk diet supplemented with iron, copper, cobalt and vitamins. Feeding graded levels of manganese in this diet showed that above 45 ppm of supplemental manganese, hemoglobin concentration and serum iron decreased, futhermore, levels approaching 5,000 ppm manganese caused a decreased tissue concentration of iron. In a second experiment in which anemic lambs were fed a roughage diet plus 0, 1,000 or 2,000 ppm added manganese, these workers found similar results and suggested that excess manganese interferes with iron absorption by antagonizing enzyme systems which oxidize or reduce iron at the absorption site. Hansard et al. (1960) supported this statement in an

experiment using rats receiving various levels of manganese in their diets. They reported that 500 or 1,000 ppm manganese depressed growth and decreased iron absorption.

In 1959, Matrone, Hartman and Clawson tested the influence of high levels of manganese on iron metabolism in anemic rabbits and pigs. They found that when a diet containing 2,000 ppm manganese was fed, hemoglobin formation was depressed in both pigs and rabbits. The minimum level of manganese interfering with hemoglobin formation seemed to be between 50 and 125 ppm. In addition, they found that a supplement of 400 ppm iron overcame the depressing effect of 2,000 ppm manganese. They also noted growth depression at levels of 1,250 and 2,000 ppm manganese.

Gubler et al. (1954) conducted an experiment concerned with the influence of manganese on copper metabolism in the rat. They found that four percent of manganese chloride supplemented to a basal diet decreased the copper concentration in the kidney and that a microcytic hypochromic anemia resulted. When a supplement of 0.1 percent copper sulfate was added to the diet, there was an increase in copper concentration of the kidney but the anemia still existed. It was suggested by these workers that manganese forms a complex with copper making it unavailable or blocks the action of copper containing enzyme systems involved in erythropoiesis, thereby causing the anemic condition. Bunch et al. (1963) found no effect of graded levels of 0, 1,000 and 2,000 ppm manganese on performance of pigs receiving a diet containing up to 250 ppm copper. In a similar experiment published in 1964 by these workers, these results were duplicated.

In work with the interaction of manganese and other mineral elements in ruminants, Pfander, Beck and Preston (1966) found that cobalt uptake by rumen microorganisms was depressed by high levels of dietary manganese and that high levels of cobalt plus manganese combined caused a reduction in the availability of zinc.

In recent years attempts have been made to study the practical aspects of feeding high levels of manganese to farm animals since there are several places in the world where high soil and plant concentrations of manganese exist. Liebholz et al. (1961) conducted experiments studying the effects of levels of manganese varying from 0 to 4,000 ppm on baby pig performance and tissue concentrations of the element. In no case did they find growth depression or adverse feed utilization due to high or low manganese levels. However, an increased concentration of manganese in liver, bone and hair was noted at levels of manganese above 40 ppm.

In 1960, Robinson et al. tested the manganese tolerance of feedlot cattle receiving 0, 250, 500 and 1,000 ppm manganese in a corn-soy basal diet. They found no significant differences in average daily gain or feed efficiency and no effect on hemoglobin, hematocrit or in concentrations of iron, calcium or phosphorus in the blood. They did find, however, a decrease in iron absorption and a decrease in cellulose digestion at high levels of manganese. This same group, Robinson et al. (1961), found similar results with growing calves receiving a bermudagrass hay diet supplemented with 0, 300 and 600 ppm manganese. Again they found no differences in growth, hemoglobin, hematocrit or blood levels of calcium, phosphorus, magnesium or iron. Iron absorption however was decreased and iron depletion increased at the highest levels of manganese.

Cunningham, Wise and Barrick (1962) conducted an experiment with growing Holstein calves receiving a corn-soy basal diet supplemented with 0, 818, 2,455 and 4,911 ppm manganese. They found that average daily gain, feed intake and feed efficiency were adversely affected by the two higher levels of manganese supplementation. There were no differences in hemoglobin concentration or in serum calcium, phosphorus, magnesium or alkaline phosphatase activity. However, in a similar experiment where feed intake was held constant for all lots receiving high or low manganese the results of the first experiment were not duplicated. They found no significant differences in growth or feed efficiency but found a decreased hemoglobin concentration in those animals on high manganese intakes. The results of the second experiment indicated that decreased growth under conditions of high manganese intake may be largely due to decreased feed intake. A limited study conducted by these workers with rumen volatile fatty acid production indicated that production of volatile fatty acids, especially propionic, is reduced under conditions of high manganese intake indicating a change in the microbial population of the rumen. Meghal and Nath (1964) also noted a change in the population of microorganisms in the ceacum of rats fed dietary manganese levels of 140 ppm as compared to rats fed only the basal diet with no supplemental manganese.

# CHAPTER III

## EXPERIMENT 1. INFLUENCE OF DIETARY MANGANESE LEVEL ON THE METABOLISM OF $^{54}Mn$, STABLE MANGANESE AND OTHER MINERAL ELEMENTS

The extremely variable levels of manganese in soils and common forages indicate the importance of investigating the effects of varying dietary manganese levels for ruminants. Increasing levels of manganese for ruminants have been reported to result in decreased growth, feed intake and feed efficiency (Cunningham, Wise and Barrick, 1962). It has also been demonstrated that increasing dietary manganese levels cause decreases in absorption of iron (Hartman, Matrone and Wise, 1955; and Robinson et al., 1960), calcium and phosphorus (Gallup et al., 1952) from the intestinal tract. There are very few reports, however, involving the effects of varying manganese levels in the diet upon the metabolism of manganese itself.

The object of this trial was to investigate the metabolism and distribution of $^{54}Mn$, a radioactive isotope of manganese, and stable manganese as influenced by dietary intake of manganese and pathway of radioisotope administration in lambs. Nutritional interrelationships of manganese with other mineral elements were also studied.

## Experimental Procedure

Sixteen Florida Native wether lambs with an average body weight of 34 kilograms were randomly allotted to a 2 x 2 factorially designed trial. One hundred microcuries of $^{54}Mn$, a radioactive isotope of manganese with a half-life of 303 days, as manganese chloride was administered either orally or intravenously into lambs being fed the basal diet shown in Table 1 containing 30 ppm manganese naturally or receiving the basal diet plus 4,000 ppm added manganese supplied from reagent grade manganese carbonate supplemented at the expense of corn starch.

The animals were housed in raised metabolism crates from the beginning of the trial and fed 900 grams of their respective experimental diet daily. Refused feed was weighed back each day prior to feeding, and tap water containing 0.05 ppm manganese was provided *ad libitum* throughout the experiment.

A 21-day preliminary feeding period was followed by the administration of the respective $^{54}Mn$ dose given either orally in a gelatin capsule or injected with physiological saline into the jugular vein as described by Hansard, Comar and Plumlee (1951). Total fecal and urinary collections were then made at 24-hour intervals for a period of 21 days. Urine was collected in plastic buckets to which had been added 100 milliliters of a solution containing 25 percent hydrochloric acid. Feces were collected in canvas fecal collection bags.

After completing the collection, a period of 35 days elapsed to allow for clearance of the radioisotope from the body, after which the lambs were again dosed with $^{54}Mn$ as before. Blood samples were obtained at periodic intervals for a period of 96 hours by means of a polyvinyl catheter in the jugular vein as described by Hansard, Comar and Plumlee

TABLE 1. COMPOSITION OF BASAL DIET

| Ingredient | Percent |
|---|---|
| Bermudagrass hay[1] | 10.00 |
| Soybean meal (50% protein) | 14.00 |
| Snapped corn[2] | 71.45 |
| Corn starch | 1.00 |
| Salt | 0.50 |
| Trace mineral mixture[3] | 0.05 |
| Bifos (21% phosphorus, 16% calcium) | 1.00 |
| Corn oil[4] | 2.00 |
| Vitamins A, D and E[5] | + |
| | 100.00 |

[1] Hay ground through 3/8 inch (1.6 cm.) screen in hammer mill.

[2] Snapped corn with cobs and shucks.

[3]

| Ingredients | % of Mixture | Mineral added to diet, ppm |
|---|---|---|
| Iron ($FeSO_4$) | 54.50 | 100.00 |
| Copper ($CuCl_2$) | 4.20 | 10.08 |
| Cobalt ($CoCO_3$) | 1.01 | 2.47 |
| Zinc ($ZnCO_3$) | 19.20 | 49.92 |
| Iodine (KI) | 1.41 | 5.43 |
| Corn Starch | 19.68 | --- |
| | 100.00 | |

[4] Stabilized with Santoquin at a level of 56 ml. per 5 gallons or 0.0297% of total diet.

[5] The following vitamins were added per pound of diet:
2000 IU - Vitamin A palmitate
270 IU - Vitamin $D_2$
5 mg. - DL alpha tocopherol

(1951). All animals were then killed and selected tissues, including liver, spleen, heart, kidney, brain, bone and muscle, removed for analysis.

Daily fecal and urinary samples as well as tissue and plasma samples were assayed for $^{54}Mn$ content in a sodium iodide crystal well counter and a large capacity 4-pi scintillation counter (arm counter). Samples were prepared especially for radioactivity determinations in order to maintain consistent geometry within the counter. Samples of fresh feces in the amount of 400 grams were taken daily from the fecal collection from each animal and compressed to a constant volume. Of the measured volume of urine excreted daily by each animal, one liter was saved and if urinary excretion was less than one liter, then it was diluted to this volume. Blood samples were centrifuged and four milliliters of plasma saved for determination of radioactivity. Tissue samples were thoroughly washed in tap water before determinations of $^{54}Mn$ content.

After radioactivity determinations, the pooled fecal collection from the last seven days of the collection period for each animal was dried in a forced-air drying oven at 70° Centrigrade. The feces were then allowed to equilibrate with room air moisture, weighed and a randomly selected aliquot saved for chemical analysis. Urine samples were saved in proportion to the daily urinary excretion for each animal from the last seven days of the collection period. Each sample was then stirred, filtered through Whatman No. 42 filter paper and approximately 100 milliliters kept for chemical analysis.

Dry matter and ash determinations were made on duplicate feed and feces samples according to the method outlined by A.O.A.C. (1960). Determinations for manganese, iron, copper, zinc, calcium, and magnesium

in feed, feces and tissue, and for manganese in urine and plasma were made by atomic absorption spectrophotometry according to the methods recommended by the manufacturer (Anonymous, 1964). Phosphorus content of feed, feces and tissue was determined by the colorimetric method outlined by Fiske and Subbarow (1925). The data were analyzed statistically by analysis of variance for factorial experiments with single degree of freedom comparisons (Steel and Torrie, 1960).

## Results

### Absorption, Excretion, Blood Clearance and Deposition of $^{54}Mn$

Table 2 shows the accumulated percent of the $^{54}Mn$ dose excreted by way of the feces on the seventh, fourteenth and twenty-first days after dosing. Dietary manganese level and pathway of isotope administration both had significant ($P < .01$ and $P < .05$, respectively) effects on the percent of the dose excreted in the feces by the seventh day. Those lambs fed only the basal diet had 77.9 percent of the dose appearing in the feces as compared to 89.2 percent for those fed supplemental manganese. Intravenous dosing also resulted in less excretion of the radioisotope by the seventh day than oral dosing, 79.3 and 87.7 percent, respectively. Pathway of isotope administration ceased to have a significant effect upon the percent of the $^{54}Mn$ dose excreted in the feces by the fourteenth day after dosing. However, dietary manganese level continued to exert a significant ($P < .05$) effect until the twenty-first day.

Figure 1 shows graphically the accumulated percent of the $^{54}Mn$ dose excreted in the feces with time. Even though dietary manganese level had a significant effect on the accumulated fecal excretion of $^{54}Mn$ over the

TABLE 2. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON ACCUMULATED PERCENT OF THE $^{54}Mn$ DOSE EXCRETED IN THE FECES

| | Dietary Manganese Level | Isotope Administration Pathway | 7 Days | 14 Days | 21 Days |
|---|---|---|---|---|---|
| Main Effects[1] | | | Percent | | |
| | Low | - | $77.9^{a}$ | $82.2^{x}$ | $83.7^{x}$ |
| | High | - | $89.2^{b}$ | $90.1^{y}$ | $90.3^{y}$ |
| | - | IV | $79.3^{x}$ | 83.5 | 85.1 |
| | - | Oral | $87.7^{y}$ | 88.8 | 89.0 |
| Simple Effects[2] | | | | | |
| | Low | IV | $70.0^{a}$ | $77.5^{x}$ | $80.3^{x}$ |
| | Low | Oral | $85.9^{b}$ | $87.0^{y}$ | $87.2^{x,y}$ |
| | High | IV | $88.8^{b}$ | $89.6^{y}$ | $89.9^{y}$ |
| | High | Oral | $89.6^{b}$ | $90.7^{y}$ | $90.8^{y}$ |

[1] Values listed are means of 8 observations.

[2] Values listed are means of 4 observations.

$^{a,b}$ Means within the same column and group with different superscripts are significantly ($P < .01$) different.

$^{x,y}$ Means within the same column and group with different superscripts are significantly ($P < .05$) different.

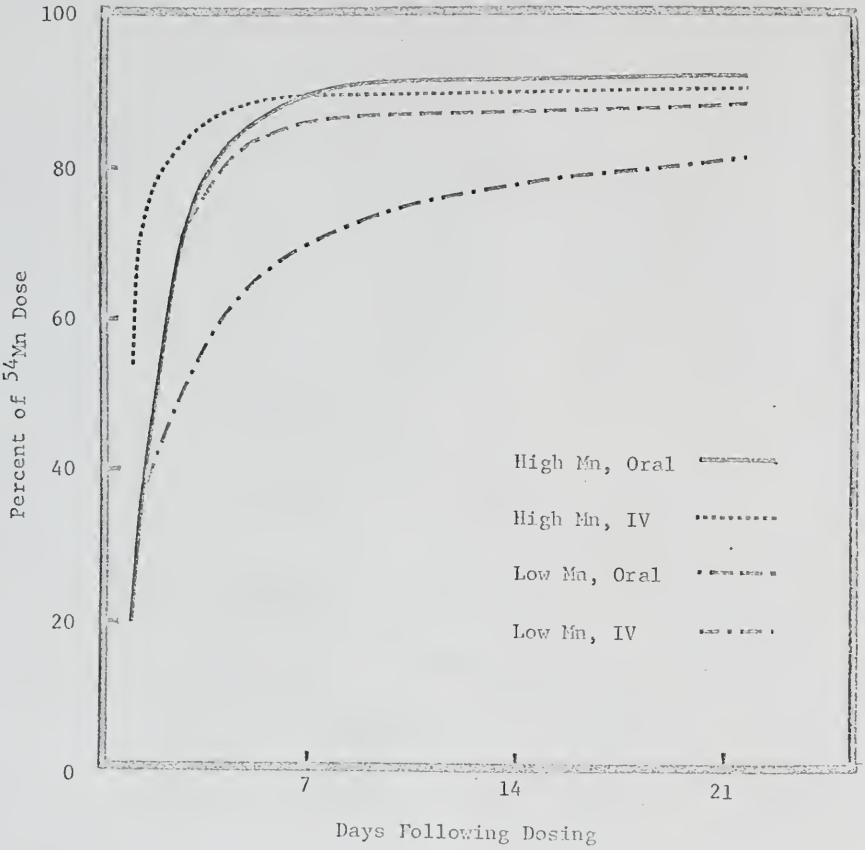


Figure 1. Accumulated Percent of $^{54}Mn$ Dose Excreted in the Feces.

entire 21-day collection period, the level of significance decreased with time. The simple effects shown on this graph (Figure 1) and in Table 2 demonstrate that lambs fed the low manganese diet and given the intravenous radioisotope dose were significantly lower than all other groups in accumulated fecal excretion of the nuclide early in the collection period. However, by day 21 they were not significantly lower than those fed the low manganese diet and given the oral dose.

The accumulated percent of the $^{54}Mn$ dose excreted in the urine by the seventh and twenty-first days after dosing is shown in Table 3. Urinary $^{54}Mn$ accounted for only a very small portion of the total excretion of the isotope, always less than 1 percent of the daily excretion. Level of dietary manganese did not significantly effect urinary excretion of the radioisotope. Pathway of isotope administration, however, did have a significant ($P < .01$) effect throughout the entire collection period. Values of 0.29 and 0.32 percent at 7 and 21 days were recorded for those given the intravenous dose compared to 0.04 and 0.06 percent for those receiving the oral dose.

The percent of the $^{54}Mn$ dose excreted in the urine with time is depicted graphically in Figure 2. As stated earlier intravenous dosing resulted in a significantly higher percent of the dose appearing in the urine regardless of dietary manganese level. Even though there was no statistically significant effect of the manganese level in the diet, those animals receiving the high dietary level had consistently more urinary $^{54}Mn$ excretion than animals dosed by the same pathway but fed the lower dietary level of manganese.

Figure 3 shows graphically the percent of the $^{54}Mn$ dose remaining in the plasma of those lambs receiving the intravenous injection of 100

TABLE 3. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON ACCUMULATED PERCENT OF THE $^{54}Mn$ DOSE EXCRETED IN THE URINE

| Dietary Manganese Level | Isotope Administration Pathway | 7 Days | 21 Days |
|---|---|---|---|
| Main Effects[1] | | | |
| Low | | 0.14 | 0.17 |
| High | | 0.18 | 0.21 |
| | IV | 0.29[a] | 0.32[a] |
| | Oral | 0.04[b] | 0.06[b] |
| Simple Effects[2] | | | |
| Low | IV | 0.25[a] | 0.29[a] |
| Low | Oral | 0.03[b] | 0.05[b] |
| High | IV | 0.32[a] | 0.35[a] |
| High | Oral | 0.04[b] | 0.06[b] |

[1] Values listed are means of 8 observations.

[2] Values listed are means of 4 observations.

[a,b] Means in the same column and group with different superscripts are significantly ($P < .01$) different.

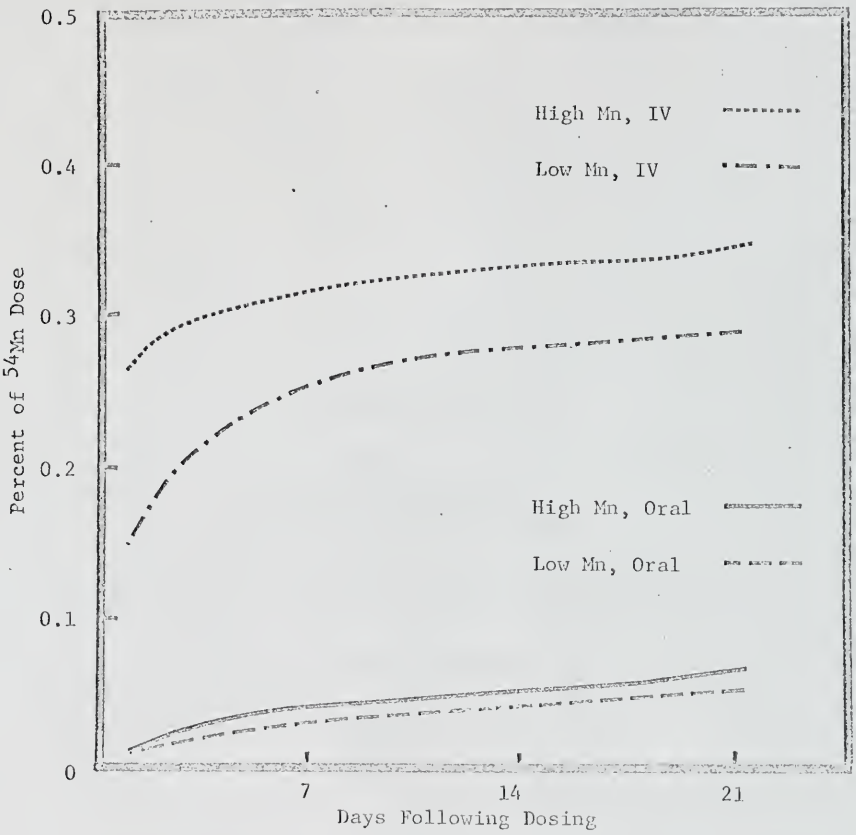


Figure 2. Accumulated Percent of $^{54}Mn$ Dose Excreted in the Urine.

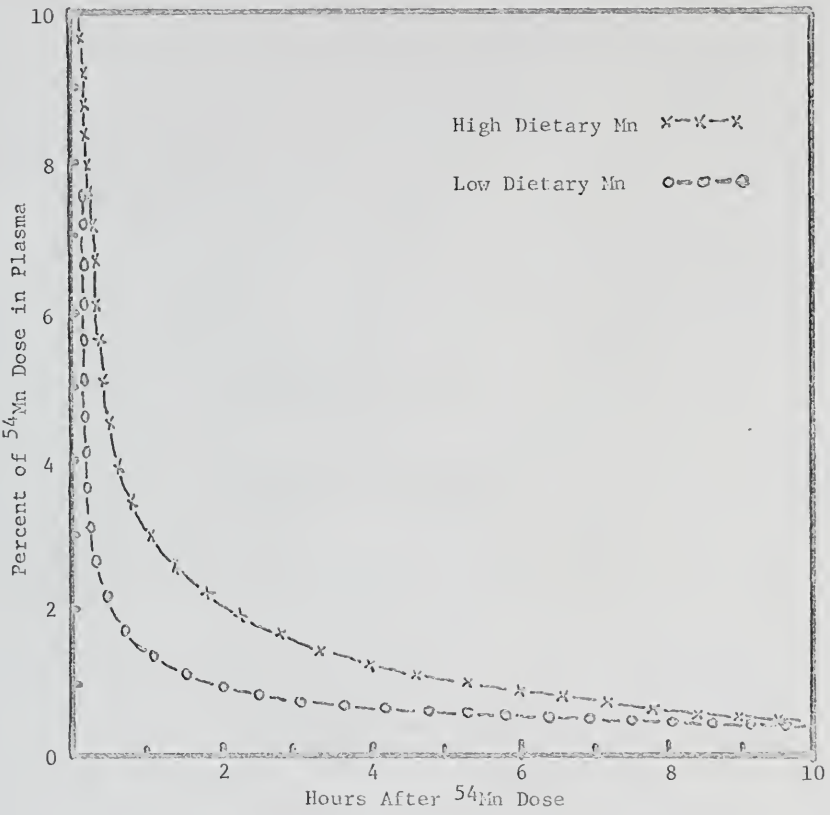


Figure 3. Plasma $^{54}Mn$ Levels of Lambs Fed Different Levels of Dietary Manganese After IV $^{54}Mn$ Dose.

microcuries from time zero to ten hours after dosing. The portion of the dose retained in the plasma decreased very rapidly to less than 1 percent. This graph indicates that lambs fed high levels of dietary manganese retained the radioactivity longer than those fed the low manganese basal diet. However, variation was quite high within groups and there were no significant differences in percent of dose retained at any point along the two lines shown. Measurable quantities of the intravenous $^{54}Mn$ dose remained in the plasma for 12 hours after dosing. However, from 12 to 96 hours, only traces of activity remained. Radioactivity in the plasma of those lambs given the oral dose of 100 microcuries of $^{54}Mn$ was never detected in more than trace quantities.

Treatment effects on tissue retention of radioactivity 96 hours after dosing are shown in Table 4. There was a highly significant ($P < .01$) manganese-level x pathway-of-isotope-administration interaction for all tissues analyzed; therefore only simple effects or individual treatment comparisons will be discussed. The average percent of the dose retained in all tissues tested was significantly ($P < .01$) greater for those lambs fed the low manganese basal diet and given the intravenous dose of the radioisotope. Retention values for this group ranged from a high of 49.9 percent retained per kilogram of dry tissue in the kidney to 1.62 percent in the muscle. There were no significant differences among the other three treatment groups in the percent of dose retained in the kidney, liver, spleen, heart or muscle. However, those animals fed the high manganese diet and given the intravenous $^{54}Mn$ dose exhibited intermediate retention values in the brain and bone.

Tissue levels of radioactivity are expressed as specific activity, or counts per minute per microgram of stable manganese, in Table 5.

TABLE 4. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON TISSUE RETENTION OF RADIOACTIVITY

| Dietary Manganese Level | Isotope Administration Pathway | Tissue | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Kidney | Liver | Spleen | Heart | Brain | Bone | Muscle |
| | | (Percent of dose per Kg. of dry tissue) | | | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | $26.03^{a}$ | $7.30^{a}$ | $6.27^{a}$ | $2.57^{a}$ | $3.70^{a}$ | 1.14 | $0.85^{a}$ |
| High | | $0.65^{b}$ | $0.35^{b}$ | $0.13^{b}$ | $0.14^{b}$ | $1.29^{b}$ | 0.74 | $0.06^{b}$ |
| | IV | $25.47^{a}$ | $6.23^{a}$ | $5.97^{a}$ | $2.58^{a}$ | $4.83^{a}$ | $1.81^{a}$ | $0.86^{a}$ |
| | Oral | $1.20^{b}$ | $1.42^{b}$ | $0.42^{b}$ | $0.13^{b}$ | $0.16^{b}$ | $0.07^{b}$ | $0.04^{b}$ |
| Simple Effects[2] | | | | | | | | |
| Low | IV | $49.92^{a}$ | $12.06^{a}$ | $11.77^{a}$ | $4.93^{a}$ | $7.22^{a}$ | $2.21^{a}$ | $1.62^{a}$ |
| Low | Oral | $2.13^{b}$ | $2.55^{b}$ | $0.77^{b}$ | $0.21^{b}$ | $0.17^{c}$ | $0.08^{c}$ | $0.07^{b}$ |
| High | IV | $1.02^{b}$ | $0.41^{b}$ | $0.17^{b}$ | $0.23^{b}$ | $2.43^{b}$ | $1.40^{b}$ | $0.10^{b}$ |
| High | Oral | $0.28^{b}$ | $0.30^{b}$ | $0.08^{b}$ | $0.05^{b}$ | $0.15^{c}$ | $0.07^{c}$ | $0.02^{b}$ |

[1] Values listed are means of 8 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

[2] Values listed are means of 4 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

TABLE 5. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON THE SPECIFIC ACTIVITY IN TISSUE

| Dietary Manganese Level | Isotope Administration Pathway | Tissue | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Kidney | Liver | Spleen | Heart | Brain | Bone | Muscle |
| | | (cpm per ug. Mn) | | | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | 2808.2[a] | 403.1[a] | 1620.1[a] | 588.5[a] | 773.5[a] | 119.5[a] | 408.5[a] |
| High | | 17.8[b] | 4.6[b] | 11.6[b] | 14.8[b] | 53.6[b] | 29.2[b] | 24.8[b] |
| | IV | 2727.7[a] | 322.6[a] | 1532.1[a] | 576.1[a] | 810.5[a] | 143.1[a] | 410.3[a] |
| | Oral | 98.3[b] | 85.2[b] | 99.6[b] | 27.1[b] | 16.7[b] | 5.6[b] | 23.1[b] |
| Simple Effects[2] | | | | | | | | |
| Low | IV | 5426.9[a] | 639.9[a] | 3046.4[a] | 1127.9[a] | 1519.1[a] | 230.9[a] | 776.2[a] |
| Low | Oral | 189.6[b] | 166.5[b] | 193.8[b] | 49.1[b] | 28.0[b] | 8.2[b] | 40.9[b] |
| High | IV | 28.5[b] | 5.3[b] | 17.9[b] | 24.4[b] | 101.8[b] | 55.3[b] | 44.4[b] |
| High | Oral | 7.1[b] | 3.9[b] | 5.3[b] | 5.2[b] | 5.4[b] | 3.0[b] | 5.3[b] |

[1] Values listed are means of 8 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

[2] Values listed are means of 4 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

There was again a highly significant manganese-level x pathway-of-isotope-administration interaction, therefore, only individual treatment comparisons will be discussed. Numeric trends were very similar, and identical in most cases, to those for percent of dose retained per kilogram of dry tissue (Table 4). Single degree of freedom treatment comparisons revealed that the combination of low dietary manganese and an intravenous radioisotope dose resulted in a significantly ($P < .01$) greater specific activity than that obtained with all other treatments for any tissue tested. There were no significant differences in specific activity among the other three treatments for any tissue.

Absorption, Excretion and Blood Levels of Stable Manganese

The data for dietary intake, fecal and urinary excretion of manganese and the calculated values for apparent absorption and net retention of the element are summarized in Table 6. An increase in intake of manganese from 21.44 to 3,393.36 milligrams per day resulted in significant ($P < .01$) increases in fecal and urinary excretion of the element from 22.85 and 0.35 milligrams to 3,573.93 and 1.47 milligrams, respectively. There were no significant differences in apparent absorption or net retention due to treatment with average values for absorption and retention being negative in every case.

Plasma levels of manganese (Table 7) were significantly ($P < .01$) higher for those lambs fed the higher levels of dietary manganese than those fed only the basal diet, with an average value of 22.24 and 16.14 micrograms per 100 milliliters, respectively. There was no significant treatment effect on hematocrit values (Table 7), the average for all animals being 45.07 percent.

TABLE 6. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON DAILY ABSORPTION AND EXCRETION OF STABLE MANGANESE

| Dietary Manganese Level | Isotope Administration Pathway | Manganese Intake (mg per day) | Fecal Manganese (mg per day) | Urinary Manganese (mg per day) | Apparent Absorption (%) | Net Retention (%) |
|---|---|---|---|---|---|---|
| Main Effects[1] | | | | | | |
| Low | | 21.44 | 22.85[a] | 0.35[a] | -6.59 | -8.24 |
| High | | 3393.36 | 3573.93[b] | 1.47[b] | -5.34 | -5.38 |
| | IV | 1714.79 | 1792.37 | 0.92 | -6.13 | -6.92 |
| | Oral | 1700.01 | 1804.41 | 0.90 | -5.79 | -6.70 |
| Simple Effects[2] | | | | | | |
| Low | IV | 21.69 | 23.37[a] | 0.33[a] | -7.77 | -9.30 |
| Low | Oral | 21.19 | 22.33[a] | 0.37[a] | -5.41 | -7.17 |
| High | IV | 3407.89 | 3561.38[b] | 1.51[b] | -4.50 | -4.55 |
| High | Oral | 3378.83 | 3586.48[b] | 1.43[b] | -6.18 | -6.22 |

[1] Values listed are means of 8 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

[2] Values listed are means of 4 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

TABLE 7. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON HEMATOCRIT AND PLASMA MANGANESE VALUES

| Dietary Manganese Level | Isotope Administration Pathway | Hematocrit (%) | Plasma Manganese (µg %) |
|---|---|---|---|
| Main Effects[1] | | | |
| Low | | 44.25 | 16.14[a] |
| High | | 46.03 | 22.24[b] |
| | IV | 43.75 | 20.05 |
| | Oral | 46.46 | 18.32 |
| Simple Effects[2] | | | |
| Low | IV | 43.42 | 16.36[a] |
| Low | Oral | 45.08 | 15.91[a] |
| High | IV | 44.08 | 23.75[b] |
| High | Oral | 47.50 | 20.74[b] |

[1] Values listed are means of 8 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

[2] Values listed are means of 4 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

Apparent Digestion and Tissue Deposition of Dietary Constituents

Average coefficients for apparent digestion of dry matter and organic matter and for apparent absorption of ash or total dietary minerals are shown in Table 8. Digestion of organic matter and dry matter was not significantly affected by treatment. The apparent absorption of ash was significantly ($P < .01$) higher for those lambs fed the low manganese diet averaging 47.7 percent compared to an average of 37.5 percent for those receiving supplemental manganese.

Apparent absorption coefficients for iron, copper, zinc, magnesium, calcium and phosphorus are listed in Table 9. Absorption of copper, zinc and calcium was not affected by treatment. Low levels of dietary manganese resulted in significantly ($P < .01$) more absorption of iron and phosphorus than the higher dietary level of manganese. Average absorption values of -3.8 and 33.8 were recorded for iron and phosphorus respectively when only the basal diet was fed as compared to -47.7 and 17.2 percent absorption when supplemental manganese was supplied. There was a significant ($P < .01$) manganese level x pathway-of-radioisotope-administration interaction effect on apparent absorption of magnesium. Analyses of simple effects revealed that those lambs fed the low manganese diet and given the intravenous radioisotope dose exhibited a significantly ($P < .01$) lower absorption of magnesium than any other treatment group. There were no significant differences in magnesium absorption among the other three treatment groups.

Mean values for the mineral composition of the liver, kidney, heart, spleen, muscle, bone and brain are shown in Table 10, and values for individual lambs are shown in appendix Tables 25-31. The manganese level in all tissues tested was directly related to the level of dietary

TABLE 8. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON DIGESTIBILITY OF ORGANIC MATTER AND DRY MATTER AND APPARENT ASH ABSORPTION

| Manganese Level | Isotope Administration Pathway | Digestion Coefficients (%) Organic Matter | Digestion Coefficients (%) Dry Matter | Apparent Absorption (%) Ash |
|---|---|---|---|---|
| Main Effects[1] | | | | |
| Low | | 73.2 | 72.0 | 47.7[a] |
| High | | 75.1 | 73.3 | 37.5[b] |
| | IV | 73.8 | 72.4 | 43.1 |
| | Oral | 74.4 | 72.9 | 42.1 |
| Simple Effects[2] | | | | |
| Low | IV | 71.8 | 70.7 | 47.8[a] |
| Low | Oral | 74.5 | 73.2 | 47.6[a] |
| High | IV | 75.8 | 74.0 | 38.4[b] |
| High | Oral | 74.3 | 72.5 | 36.6[b] |

[1] Values listed are means of 8 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

[2] Values listed are means of 4 observations and means within the same column and group with different superscripts are significantly ($P < .01$) different.

TABLE 9. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON PERCENT APPARENT ABSORPTION OF MINERAL ELEMENTS BY SHEEP

| Dietary Manganese Level | Isotope Administration Pathway | Apparent Absorption (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Iron | Copper | Zinc | Magnesium | Calcium | Phosphorus |
| Main Effects[1] | | | | | | | |
| Low | | -3.8[a] | 21.0 | -59.3 | 27.4[x] | 19.8 | 33.8[a] |
| High | | -47.7[b] | 26.9 | -80.7 | 34.2[y] | 15.6 | 17.2[b] |
| | IV | -34.4 | 24.2 | -76.9 | 24.8[a] | 18.3 | 26.3 |
| | Oral | -17.2 | 23.7 | -63.2 | 36.8[b] | 17.0 | 24.7 |
| Simple Effects[2] | | | | | | | |
| Low | IV | -3.1[a] | 22.1 | -38.6 | 13.6[a] | 22.8 | 32.5[a] |
| Low | Oral | -4.5[a] | 19.9 | -80.1 | 41.1[b] | 16.9 | 35.2[a] |
| High | IV | -65.6[b] | 26.2 | -115.1 | 35.9[b] | 13.9 | 20.2[b] |
| High | Oral | -29.9[b] | 27.6 | -46.3 | 32.5[b] | 17.2 | 14.2[b] |

[1] Values listed are means of 8 observations.

[2] Values listed are means of 4 observations.

[a,b] Means within the same column and group with different superscripts are significantly ($P < .01$) different.

[x,y] Means within the same column and group with different superscripts are significantly ($P < .05$) different.

TABLE 10. MAIN AND SIMPLE EFFECTS OF DIETARY MANGANESE LEVEL AND PATHWAY OF RADIOISOTOPE ADMINISTRATION ON TISSUE MINERAL COMPOSITION OF SHEEP

| Dietary Mn Level | Isotope Admin. Pathway | Tissue Minerals, ppm in Dry Matter | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Mg | Ca | P |
| | | | | Liver | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | 9.9[a] | 1055[x] | 42.8[a] | 193[a] | 588 | 163 | 11,743 |
| High | | 44.2[b] | 698[y] | 62.2[b] | 166[b] | 608 | 157 | 12,059 |
| | IV | 25.8 | 868 | 55.3 | 193[x] | 606 | 159 | 11,727 |
| | Oral | 28.2 | 885 | 49.7 | 167[y] | 590 | 161 | 12,050 |
| Simple Effects[2] | | | | | | | | |
| Low | IV | 9.7[a] | 1021[x] | 49.7[a] | 211[a] | 588 | 157 | 11,110 |
| Low | Oral | 10.0[a] | 1090[x] | 35.9[a] | 175[b] | 587 | 168 | 12,325 |
| High | IV | 42.0[b] | 715[y] | 61.0[b] | 175[b] | 623 | 161 | 12,344 |
| High | Oral | 46.4[b] | 681[y] | 63.4[b] | 158[b] | 592 | 154 | 11,774 |
| | | | | Kidney | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | 5.2[a] | 268 | 17.4 | 141 | 739 | 399 | 11,545 |
| High | | 22.5[b] | 224 | 18.9 | 165 | 780 | 403 | 11,838 |
| | IV | 13.0 | 260 | 19.3 | 159 | 762 | 409 | 11,676 |
| | Oral | 14.6 | 232 | 17.1 | 147 | 757 | 394 | 11,723 |
| Simple Effects[2] | | | | | | | | |
| Low | IV | 4.4[a] | 277 | 18.6 | 149 | 736 | 412 | 10,693 |
| Low | Oral | 6.0[a] | 259 | 16.3 | 133 | 742 | 386 | 12,184 |
| High | IV | 21.7[b] | 243 | 19.9 | 169 | 789 | 405 | 12,413 |
| High | Oral | 23.2[b] | 205 | 17.9 | 161 | 771 | 402 | 11,262 |
| | | | | Heart | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | 2.4[a] | 283 | 16.8 | 85 | 895 | 190 | 9,683 |
| High | | 5.4[b] | 272 | 17.6 | 86 | 898 | 198 | 10,034 |
| | IV | 3.8 | 276 | 16.8 | 86 | 875 | 197 | 9,564 |
| | Oral | 4.0 | 279 | 17.6 | 86 | 918 | 192 | 10,152 |

TABLE 10. (continued)

| Dietary Mn Level | Isotope Admin. Pathway | Tissue Minerals, ppm in Dry Matter | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Mg | Ca | P |
| Simple Effects[2] | | | | | | | | |
| Low | IV | 2.3[a] | 271 | 16.6 | 86 | 885 | 202 | 9,439 |
| Low | Oral | 2.5[a] | 294 | 17.1 | 85 | 905 | 178 | 9,927 |
| High | IV | 5.2[b] | 281 | 17.0 | 85 | 864 | 192 | 9,690 |
| High | Oral | 5.5[b] | 264 | 18.2 | 87 | 931 | 205 | 10,376 |
| | | | | Spleen | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | 2.6[a] | 4423 | 9.3 | 141 | 752 | 225 | 12,701 |
| High | | 6.4[b] | 3056 | 8.2 | 134 | 717 | 212 | 12,972 |
| | IV | 3.6 | 3272 | 8.1 | 133 | 735 | 223 | 13,341 |
| | Oral | 5.3 | 4207 | 9.4 | 142 | 734 | 214 | 12,378 |
| Simple Effects[2] | | | | | | | | |
| Low | IV | 2.0[a] | 4238 | 8.9 | 139 | 762 | 241 | 12,787 |
| Low | Oral | 3.1[a] | 4608 | 9.6 | 143 | 742 | 210 | 12,614 |
| High | IV | 5.3[b] | 2306 | 7.2 | 127 | 708 | 206 | 14,079 |
| High | Oral | 7.5[b] | 3807 | 9.3 | 140 | 727 | 218 | 12,142 |
| | | | | Muscle | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | 1.0[x] | 130 | 5.9 | 126 | 802 | 124 | 7,345 |
| High | | 1.6[y] | 120 | 4.9 | 120 | 842 | 127 | 7,971 |
| | IV | 1.2 | 121 | 5.4 | 124 | 847 | 128 | 7,652 |
| | Oral | 1.4 | 129 | 5.5 | 122 | 798 | 123 | 7,663 |
| Simple Effects[2] | | | | | | | | |
| Low | IV | 1.1[x] | 139 | 7.0 | 118 | 839 | 126 | 7,348 |
| Low | Oral | 0.9[x] | 121 | 4.9 | 133 | 766 | 121 | 7,342 |
| High | IV | 1.3[y] | 103 | 3.8 | 129 | 855 | 131 | 7,958 |
| High | Oral | 1.9[y] | 138 | 6.1 | 112 | 829 | 124 | 7,983 |

TABLE 10. (continued)

| Dietary Mn Level | Isotope Admin. Pathway | Tissue Minerals, ppm in Dry Matter | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Mg | Ca | P |
| | | | | Bone | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | 5.2[a] | 34 | 2.8[a] | 321 | 15,484 | 312,166[x] | 180,796 |
| High | | 14.3[b] | 34 | 4.1[b] | 325 | 14,820 | 318,867[y] | 175,396 |
| | IV | 10.1 | 32 | 3.1[x] | 320 | 16,299[a] | 317,659 | 175,617 |
| | Oral | 9.4 | 36 | 3.8[y] | 325 | 14,005[b] | 313,374 | 180,575 |
| Simple Effects[2] | | | | | | | | |
| Low | IV | 5.0[a] | 33 | 2.4[x] | 344 | 16,323 | 315,107[x] | 178,072 |
| Low | Oral | 5.4[a] | 34 | 3.2[y] | 297 | 14,646 | 309,226[x] | 183,521 |
| High | IV | 15.1[b] | 31 | 3.8[y] | 295 | 16,275 | 320,212[y] | 173,161 |
| High | Oral | 13.4[b] | 37 | 4.5[y] | 354 | 13,365 | 317,522[y] | 177,630 |
| | | | | Brain | | | | |
| Main Effects[1] | | | | | | | | |
| Low | | 3.1[a] | 185 | 14.5 | 73 | 643 | 308 | 15,721 |
| High | | 14.6[b] | 158 | 15.5 | 68 | 632 | 305 | 15,280 |
| | IV | 8.7 | 172 | 14.5 | 72 | 647 | 306 | 15,431 |
| | Oral | 9.0 | 171 | 15.4 | 69 | 628 | 307 | 15,423 |
| Simple Effects[2] | | | | | | | | |
| Low | IV | 2.9[a] | 185 | 14.0 | 73 | 652 | 306 | 15,952 |
| Low | Oral | 3.4[a] | 185 | 14.9 | 72 | 634 | 309 | 15,491 |
| High | IV | 14.6[b] | 159 | 14.9 | 71 | 642 | 305 | 15,170 |
| High | Oral | 14.6[b] | 156 | 16.0 | 66 | 622 | 304 | 15,390 |

[1] Each value based on 8 observations per treatment for main effects.

[2] Each value based on 4 observations per treatment for simple effects.

[a,b] Means within the same column and group with different superscripts are significantly ($P < .01$) different.

[x,y] Means within the same column and group with different superscripts are significantly ($P < .05$) different.

manganese fed. Manganese levels were greater in the liver, kidney, heart, spleen, bone, brain ($P < .01$) and muscle ($P < .05$) when the high manganese diet was fed. The high manganese diet resulted in significantly lower levels of iron ($P < .05$), copper and zinc ($P < .01$) in the liver. However, bone levels of copper ($P < .01$) and calcium ($P < .05$) were significantly increased by high dietary manganese levels.

Pathway of isotope administration had a significant effect on the tissue concentration of three minerals. Those lambs given the oral dose of $^{54}Mn$ had significantly lower levels of zinc in the liver ($P < .05$) and less magnesium in bone ($P < .01$), but higher levels of bone copper ($P < .05$) than those given $^{54}Mn$ intravenously.

## Discussion

The results of this study demonstrating that both $^{54}Mn$ and stable manganese are excreted almost exclusively by way of the feces are in agreement with previous research findings by this author (Watson, 1968a), Cotzias (1958), and others. The increase in excretion of the radioisotope and stable manganese with increased dietary manganese was expected due to mass action. With excretion primarily fecal, the finding that the body retained an intravenously injected dose of $^{54}Mn$ for a longer period of time than an oral dose of the radioisotope was also in accord with expectations.

The extremely rapid plasma disappearance rate of intravenously injected $^{54}Mn$ is in agreement with similar studies conducted with rats (Cotzias, 1963). The body seems to have a mechanism for homeostatic control, maintaining low blood levels of manganese and other trace elements. Even though the plasma concentration of stable manganese was

greater for those lambs fed the higher level of dietary manganese, the values recorded for both groups were very close to the "normal" range reported by Underwood in 1962 (5 to 20 micrograms percent).

Apparent absorption and net retention values (Table 6) for stable manganese and tissue radioisotope levels in orally dosed animals (Tables 4 and 5) indicate that absorption of manganese from the intestinal tract is limited. This agrees with reports by Skinner, Peterson and Steenbock (1931) using rats and by Lassiter and White (1966) using sheep. The negative treatment averages for percent absorption and retention of stable manganese (Table 6) are difficult to explain. However, these findings are in agreement with previous research by this author (Watson, 1968a) and by Lassiter (1970). In this experiment both positive and negative values for absorption and retention were recorded, but fecal manganese was approximately equal to dietary intake of the element. Variation was also quite high within all treatments. Skinner, Peterson and Steenbock (1931) reported that rats excrete as much as 99 percent of their dietary intake in the feces. Even though negative balances were recorded, tissue deposition of both stable manganese and $^{54}Mn$ demonstrates that manganese is absorbed from the intestinal tract. The inverse relationship between dietary manganese level and the percent of the radioactive manganese dose retained by various tissue is in agreement with research with rats (Rojas, Dyer and Cassatt, 1966), chickens (Settle et al., 1969) and swine (Plumlee et al., 1956). The relative enrichment of tissues with radioactivity in this experiment is also in general agreement with the results reported by these researchers. As was noted by Cotzias (1963), those tissue which are richest in concentration of mitochondria (liver and kidney) retain more manganese. This

supports the theory that the functional activity of manganese may be located in the mitochondria.

The decreased apparent absorption of ash or total minerals in those lambs fed supplemental manganese is partially explained not only by the limited absorption of manganese itself, but by the decrease in absorption of iron and phosphorus resulting from the addition of manganese. These results are supported by the finding of Gallup et al. (1952) with steers, and Hartman, Matrone and Wise (1955) with lambs that excess manganese interferes with absorption of phosphorus and iron.

The absorption values for the dietary minerals in this experiment are similar to, but slightly lower in most cases, than values obtained by Standish (1970) with sheep fed an experimental diet similar in composition to the one used here. The negative absorption of iron and zinc as well as the individual treatment differences in magnesium absorption may be due to intakes of these elements from extraneous sources. The animals were housed in metal metabolism cages and were periodically observed chewing on the cages. Similar results have been observed with lambs fed in the same cages in the past (Standish, 1970 and Watson, 1968b).

The increased concentration of manganese in the tissue of lambs fed the higher level of dietary manganese is supported by the work of Rojas, Dyer and Cassatt (1965) with cattle, Johnson (1943) and Svajgr, Peo and Vipperman (1969) with swine and Mathers and Hill (1968) with poultry. The manganese concentration in the tissue of the lambs fed the basal diet is very similar to "normal" levels reported by the above authors as well as Loaiza (1968) and Standish (1970).

Tissue concentrations of all the mineral elements recorded in this

experiment are similar to values reported by Loaiza (1968) with pasture-fed cattle and by Standish (1970) with cattle fed a diet similar in composition to the diet used in the present studies.

The decreased level of iron in the liver as a result of increased dietary manganese agrees with results of Hartman, Matrone and Wise (1955). Also, the lower zinc level in the livers of those lambs fed the high manganese diet demonstrates the antagonistic effect of excess manganese on zinc utilization reported by Pfander, Beck and Preston (1966). The increased concentrations of copper in the liver and bone and increased bone calcium resulting from increased dietary manganese are in contrast to the findings of Gallup et al. (1952) and Gubler et al. (1954). This author is unable to attach biological significance to the effect of pathway of radioisotope administration on levels of zinc in the liver and magnesium and copper levels in the bone since such a small quantity of manganese was administered in the radioactive dose. No such effects have been reported by other researchers using radioisotopes of manganese.

## Summary

Sixteen Florida Native wether lambs were employed in a 2 x 2 factorially designed trial to study the metabolism and distribution of manganese as well as some of the nutritional interrelationships which are exhibited by the element. One hundred microcuries of $^{54}Mn$, a radioisotope of manganese, were administered either orally or intravenously to lambs receiving a basal diet based on hay, corn and soybean meal and containing 30 ppm manganese, or the basal diet plus 4,000 ppm supplemental manganese. A 21-day fecal and urinary collection period

followed dosing. A 35-day clearance period elapsed before the lambs were again dosed with $^{54}Mn$. Blood samples were then taken at periodic intervals for 96 hours after which all animals were killed and selected tissues removed for analyses. Absorption of manganese from the intestinal tract appeared to be low and excretion of both stable manganese and $^{54}Mn$ was almost exclusively by way of the feces regardless of dietary manganese level or pathway of isotope administration. Low levels of dietary manganese and intravenous administration of the radioisotope resulted in the greatest tissue retention of radioactivity. The tissues, listed in decreasing order of enrichment with radioactive manganese were as follows: kidney, liver, spleen, brain, heart, bone and muscle. Tissue and plasma levels of stable manganese were greater for those lambs fed the manganese-supplemented diet than for those fed the unsupplemented basal diet. The high manganese diet also resulted in decreased absorption of iron and phosphorus from the intestinal tract and decreased liver concentrations of iron and zinc.

## CHAPTER IV

## EXPERIMENTS 2 AND 3. BIOLOGICAL ASSAY OF INORGANIC MANGANESE FOR CHICKS

Many inorganic forms of manganese are available for use as supplements in poultry feeds. A suitable assay for biological availability of the element, however, is not available, and little is known of the relative utilization of the various supplemental sources by the chick. Early reports by Schaible, Bandemer and Dividson (1938) and Gallup and Norris (1939a) stated that practically all the chemical forms were of equal value to the chick except for a few carbonate and silicate ores. In these studies, the only criterion of availability was the ability of the manganese supplement to prevent the occurrence of perosis. More recently, Hennig et al. (1967) reported that radioactive manganese as $^{54}MnCl_2$ was incorporated into the body of a chick to a significantly greater extent than manganese as $^{54}MnSO_4$ or $^{54}MnO_2$. Only a few of the products which are currently available for use as manganese supplements were tested, and the levels of manganese added to the diets were not sufficiently low to provide a marginal state of manganese nutrition in which extremely sensitive responses would be expected.

Two experiments were conducted with growing chicks receiving varied low levels of supplemental manganese from different sources. Objectives of this research were to (1) develop a suitable biological assay for

manganese and (2) determine the relative availability of manganese from certain inorganic sources.

## Experimental Procedure

The forms of manganese tested in these experiments were reagent-grade manganese sulfate ($MnSO_4 \cdot H_2O$), as the reference standard, and two commercial feed-grade manganese oxides, hereafter referred to as manganese oxide No. 1 and manganese oxide No. 2. Table 11 presents the average manganese contents of the three sources as determined by two laboratories. These values were used in preparing experimental diets. Other chemical constituents, results of X-ray diffraction studies, and relative solubilities for each of the three test materials are also shown. The solubility of each manganese material was obtained in water, 0.4 percent hydrochloric acid, 2 percent citric acid, and neutral ammonium citrate. Solubility in each solvent was determined by adding 100 milliliters of the solvent to 0.1 grams of the test material, stirring constantly for one hour at a temperature of 37 degrees Centigrade, then filtering through No. 42 filter paper. Manganese content was then determined on the filtrate by atomic absorption spectrophotometry as outlined by the manufacturer (Anonymous, 1964).

The basal diet used in both of these experiments is shown in Table 12. This semi-purified diet is similar in composition to that used by Leach, Norris and Scott (1962) in studying the relationship of choline to perosis in chicks. Those nutrients known to be related to the perosis syndrome, including inositol, biotin, choline, folic acid, vitamin $B_{12}$ and pyridroxine, were supplemented at levels well above established requirements. The diet, as formulated, contained 4 ppm manganese.

TABLE 11. CHEMICAL COMPOSITION OF MANGANESE SUPPLEMENTS, THEIR SOLUBILITY IN VARIOUS SOLVENTS AND X-RAY PATTERNS

| Supplement | Chemical Constituents, (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mn | Fe | Ca | Mg | P | Cu | Zn |
| $MnSO_4.H_2O$ | 31.16 | 0.03 | 0.002 | 0.001 | --- | 0.052 | 0.004 |
| MnO - No. 1 | 51.95 | 5.63 | 0.042 | 0.123 | 0.402 | 0.112 | 0.060 |
| MnO - No. 2 | 36.10 | 5.46 | 0.816 | 0.441 | 0.223 | 0.065 | 0.098 |

| | Solubility, (%) | | | | |
|---|---|---|---|---|---|
| | Water | 0.4% Hydrochloric acid | 2% Citric acid | Neutral ammonium citrate | Interpretation of X-ray Patterns |
| $MnSO_4.H_2O$ | 100.0 | 100.0 | 100.0 | 100.0 | $MnSO_4.H_2O$ |
| MnO - No. 1 | 0.0 | 35.4 | 77.5 | 29.1 | MnO (Manganosite) and $Mn_3O_4$ (Hausmannite) |
| MnO - No. 2 | 0.0 | 52.0 | 87.4 | 16.5 | MnO, $Mn_3O_4$, and Quartz |

TABLE 12. COMPOSITION OF BASAL DIET[1]

| Ingredient | % |
|---|---|
| Glucose | 57.97 |
| Soybean protein[2] | 27.00 |
| Corn oil | 3.00 |
| Cellulose | 3.00 |
| Vitamin mixture[3] | 2.20 |
| Mineral mixture[4] | 5.83 |
| Glycine | 0.30 |
| DL Methionine | 0.70 |
| | 100.00 |

1 Test diets contained the experimental manganese source added at the expense of glucose.

2 Assay Protein, C-1, Skidmore Enterprises, Cincinnati, Ohio.

3 Supplied the following per kg of diet: inositol, 1510 mg.; niacin, 99.2 mg.; thiamine HCl, 22.1 mg.; riboflavin, 22.1 mg.; Ca pantothenate, 66.2 mg.; pyridoxine HCl, 22.1 mg.; menadione sodium bisulfite, 49.6 mg.; vitamin A, 19,846 I.U.; vitamin $D_3$, 2205 I.U.; alpha tocopherol, 110.3 mg.; ascorbic acid, 992.3 mg.; choline chloride, 1653 mg.; p aminobenzoic acid, 110.3 mg.; biotin, 0.44 mg.; folic acid, 1.98 mg.; and vitamin $B_{12}$, 0.03 mg.

4 Supplied the following per kg of diet: $CaHPO_4$, 1.70 gm.; $CaCO_3$, 1.83 gm.; $KH_2PO_4$, 1.38 gm.; NaCl, 0.60 gm.; $MgSO_4$, 0.25 gm.; $FeSO_4 \cdot 7H_2O$, 33.30 mg.; KI, 0.26 mg.; $CuSO_4 \cdot 5H_2O$, 1.67 mg.; $CoCl_2 \cdot 6H_2O$, 0.17 mg.; $Na_2MoO_4 \cdot 2H_2O$, 0.83 mg.; and ZnO, 7.47 mg.

Experimental treatments were prepared by adding graded levels of the three manganese sources to the basal diet at the expense of glucose.

Each experiment consisted of eight treatments and each treatment included three replicates of ten chicks which were randomly assigned to experimental groups. The eight treatments tested were: basal diet, basal plus 10, 20 and 30 ppm of manganese supplied from manganese sulfate, basal plus 10 and 20 ppm from manganese oxide No. 1, and basal plus 10 and 20 ppm from manganese oxide No. 2. The levels of manganese in all diets were verified by chemical analysis.

Two-hundred-forty (240) day-old Leghorn cockerel chicks were obtained from a local commercial hatchery for each experiment. Treatment replicates were randomly assigned to pens in a thermostatically controlled, electrically heated battery brooder constructed primarily of stainless steel with raised wire floors. All chicks received their respective experimental diet and tap water (0.4 ppm manganese) ad libitum throughout the 28-day trial. After 28 days, body weights were recorded and legs of all chicks were individually examined for abnormal development or perosis. Upon visual examination, each chick was assigned a value within the range of from zero to four, depending upon the degree of abnormal leg development. A value of zero was assigned when the leg appeared completely normal. A value of one was given when there was a slight amount of swelling of the tibiometatarsal joint, two was assigned when there was a marked degree of swelling of the joint, three when there was swelling plus a slight amount of slipping of the achilles tendon, and four when swelling was combined with a marked degree of slipping of the tendon.

Chicks were sacrificed after the examination for perosis. In

Experiment 2, the right tibiae were removed from seven chicks on each treatment for determination of percent bone ash. Bone ash and bone manganese levels were determined on the combined right tibiae and femur of all 30 chicks on each treatment in Experiment 3. Breaking strength was also determined on the left tibiae of all chicks in Experiment 3 as described by Rowland et al. (1967). Bones were ashed according to the method described by A.O.A.C. (1960). Bone manganese content was determined by atomic absorption spectrophotometry.

The data for body weights, bone abnormalities, bone ash, breaking strength, and bone manganese were subjected to analysis of variance, and bone manganese data were subjected to regression and correlation analyses as outlined by Steele and Torrie (1960). Significant differences between treatment means were determined by Duncan's multiple range test (1955).

## Results

Statistical analyses of the data obtained from body weight and leg abnormality measurements revealed no experiment x treatment interactions so the data from the two experiments were combined for presentation. The results of the body weight determination at 28 days of age are shown in Table 13. Average weight of the chicks receiving the basal diet (252 grams) was lower ($P < .01$) than that of the other treatment groups. Chicks fed the basal diet with 10 ppm manganese from manganese oxide No. 2 had an average weight of 288 grams which was lower ($P < .01$) than the weight of the group receiving the basal plus 20 ppm manganese from the same source. There were no significant differences in average weights among treatment groups fed any other supplemental level or source of the element.

TABLE 13. BODY WEIGHTS OF 28-DAY-OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE (EXPERIMENTS 2 AND 3)[1]

| Manganese Source | Supplemental Manganese (ppm) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 30 |
| | | Body wt., gm | | |
| $MnSO_4.H_2O$ | $252^{a}$ | $306^{b,c}$ | $308^{b,c}$ | $317^{b,c}$ |
| MnO - No. 1 | --- | $302^{b,c}$ | $316^{b,c}$ | --- |
| MnO - No. 2 | --- | $288^{b}$ | $323^{c}$ | --- |

[1]Values listed represent an average of 60 chicks and means bearing different superscripts are significantly different ($P < .01$).

The incidence of leg abnormalities was related to the response obtained for growth rate (Table 14). The value for degree of leg abnormalities in the groups fed the basal diet was higher ($P < .01$) than for any other treatment group. Also, those chicks receiving the basal plus 10 ppm manganese from the manganese oxide No. 2 had a higher ($P < .01$) assigned value than the chicks fed any other level or source of supplemental manganese. There were no significant differences among any of the other treatment groups in the incidence of leg abnormalities.

Statistical analyses of the data obtained from bone ash determinations revealed a significant experiment x treatment interaction; thus the data from the two experiments are presented separately (Table 15). Dietary levels and source of manganese had no effect on percent bone ash of chicks in Experiment 2 when the comparison was based on the data of seven chicks per treatment. In Experiment 3, with 30 observations per treatment, bone ash response was similar to that obtained for growth rate and leg development. The treatment groups fed the basal diet and those fed the basal plus 10 ppm of the element from manganese oxide No. 2 had the lowest levels of bone ash and were not significantly different. The highest level of bone ash was found in those chicks receiving 20 ppm of the element from manganese oxide No. 2, and all other treatment groups were intermediate in bone ash response.

Treatment averages for tibiae breaking-strength determinations in Experiment 3 are presented in Table 16. The average breaking strength of the tibiae for all treatments was 10.5 pounds, and statistical analyses of these values revealed no significant differences among treatments.

Table 17 presents the average values for bone manganese for all treatments in Experiment 3. Treatment differences were more pronounced

TABLE 14. LEG ABNORMALITY SCORES OF 28-DAY-OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE (EXPERIMENTS 2 AND 3)[1,2]

| Manganese Source | Supplemental Manganese (ppm) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 30 |
| | Leg Score, (0-4) | | | |
| $MnSO_4 \cdot H_2O$ | 3.01[a] | 0.75[c] | 0.55[c] | 0.27[c] |
| MnO - No. 1 | --- | 0.76[c] | 0.66[c] | --- |
| MnO - No. 2 | --- | 1.53[b] | 0.57[c] | --- |

[1] Grading Scale: 0 = Normal leg
1 = Slight swelling of tibiometatarsal joint
2 = Marked swelling of joint
3 = Swelling and slipping of tendon
4 = Swelling and marked slipping of tendon

[2] Values listed represent an average of 120 observations and means bearing different superscripts are significantly different ($P < .01$).

TABLE 15. BONE ASH OF 28-DAY-OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE

| Manganese Source | Supplemental Manganese (ppm) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 30 |
| | Experiment 2[1] | | | |
| | % in dry, fat-free tibia | | | |
| $MnSO_4$ $H_2O$ | 47.6 | 48.3 | 46.7 | 46.3 |
| MnO - No. 1 | --- | 46.9 | 46.6 | --- |
| MnO - No. 2 | --- | 47.0 | 46.4 | --- |
| | Experiment 3[2] | | | |
| | % in dry, fat-free tibia and femur | | | |
| $MnSO_4$ $H_2O$ | $45.8^{a,b}$ | $47.4^{c,d}$ | $47.5^{c,d}$ | $46.7^{b,c,d}$ |
| MnO - No. 1 | --- | $46.2^{b,c}$ | $47.2^{c,d}$ | --- |
| MnO - No. 2 | --- | $44.7^{a}$ | $47.7^{d}$ | --- |

[1] Values listed are treatment averages based on 7 observations per treatment.

[2] Values listed are treatment averages based on 30 observations per treatment. Means with different superscripts are significantly different ($P < .01$).

TABLE 16. TIBIA BREAKING STRENGTH OF 28-DAY-OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE[1]

| Manganese Source | Supplemental Manganese (ppm) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 30 |
| | (pounds) | | | |
| $MnSO_4.H_2O$ | 11.5 | 11.8 | 9.4 | 9.4 |
| MnO - No. 1 | --- | 10.4 | 8.3 | --- |
| MnO - No. 2 | --- | 11.2 | 11.5 | --- |

[1] Values represent means of 30 observations per treatment.

TABLE 17. BONE (TIBIA PLUS FEMUR) MANGANESE LEVELS OF 28-DAY-OLD CHICKS FED VARIOUS FORMS AND LEVELS OF SUPPLEMENTAL MANGANESE[1]

| Manganese Source | Supplemental Manganese (ppm) | | | |
|---|---|---|---|---|
| | 0 | 10 | 20 | 30 |
| | (ppm in dry, fat-free bone) | | | |
| $MnSO_4 . H_2O$ | $2.46^{a}$ | $2.88^{b,c}$ | $3.30^{d}$ | $3.81^{e}$ |
| MnO - No. 1 | --- | $2.95^{b,c}$ | $3.17^{d,c}$ | --- |
| MnO - No. 2 | --- | $2.74^{b}$ | $3.39^{d}$ | --- |
| | (ppm in bone ash) | | | |
| $MnSO_4 . H_2O$ | $5.11^{a}$ | $6.08^{a,b}$ | $6.80^{a,b,c}$ | $8.17^{c}$ |
| MnO - No. 1 | --- | $6.27^{a,b}$ | $6.71^{a,b,c}$ | --- |
| MnO - No. 2 | --- | $6.12^{a,b}$ | $7.10^{a,b,c}$ | --- |

[1] Values listed are treatment averages of 30 observations and means bearing different superscripts are significantly different ($P < .05$).

when bone manganese was expressed as ppm in dry, fat-free bone rather than ppm of manganese in bone ash. The manganese level in the dry, fat-free bone of chicks fed the basal diet with no supplemental manganese (2.46 ppm) was lower ($P < .05$) than the level found in any other treatment group. For those groups receiving supplemental manganese from the standard manganese sulfate or from manganese oxide No. 2 the manganese level in dry, fat-free bone directly reflected the changes in dietary manganese levels ($P < .05$). The same numerical trend was demonstrated for those groups receiving manganese oxide No. 1. When expressed as ppm in bone ash, bone manganese levels also increased numerically in every case with increasing dietary levels of the element. The manganese level in bone ash of chicks fed only the basal diet was significantly lower ($P < .05$) than that of those receiving 30 ppm of the element from the standard manganese sulfate. Those fed 30 ppm supplemental manganese from manganese sulfate also had a higher level ($P < .05$) of the element in the bone ash than any group receiving 10 ppm of added manganese from any source.

The relationship between dietary levels of manganese as manganese sulfate and bone manganese is shown graphically in Figure 4. Significant ($P < .01$) correlations of 0.665 and 0.667 existed between manganese intake and level of bone manganese when expressed as ppm in the dry, fat-free bone or ppm in bone ash respectively. The linear regressions obtained are shown as follows: $Y = 2.28 + 0.044X$ and $Y' = 4.82 + 0.091X$ where X is equal to the dietary manganese level, Y equals the level of manganese in dry, fat-free bone, and Y' the level of manganese in bone ash.

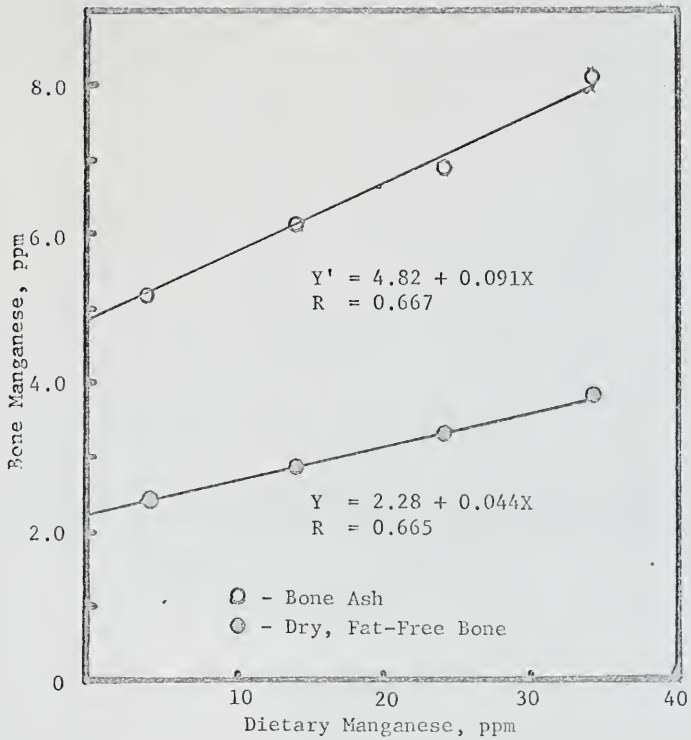


Figure 4. Relation of Bone (tibia plus femur) Manganese Levels to Manganese Intake in the Form of Manganese Sulfate.

## Discussion

These data are in agreement with those published recently by Settle et al. (1969) which showed that a similar semi-purified diet, when fed alone, was totally inadequate in supporting maximum growth rate and in preventing the occurrence of perosis. From the data obtained in the studies reported herein, it is concluded that 10 ppm manganese added to the diet used in these experiments from either the sulfate or the manganese oxide No. 1 source were adequate to promote normal growth rate and leg development. These two sources of manganese were more available to the chick than was the manganese oxide No. 2 since 10 ppm manganese from the latter source produced a lower growth rate and a higher incidence of perosis. Results of bone ash determinations are in general agreement with the results of measurements of growth rate and incidence of perosis; however, results were not as well defined. Percent bone ash was generally increased by the addition of manganese to the basal diet from each source except at the level of 10 ppm from manganese oxide No. 2.

Bone manganese levels were more directly related to dietary levels of the element than were growth rate or leg development since a significant linear increase in bone manganese resulted from increasing dietary levels of supplemental manganese as reagent-grade manganese sulfate from zero to 30 ppm (Figure 4). A significant linear response was not found with body weights (Table 13), leg abnormality scores (Table 14) or bone ash (Table 15). This indicates that levels of manganese in the bone may be a sufficiently sensitive response criterion to detect small differences in dietary levels or differences in biological availability of manganese. These results suggest that a biological assay

for manganese may be developed using manganese sulfate as a reference standard and bone manganese levels along with growth rate and leg development as the response criteria.

Growth rate and leg development were directly related to the solubilities of the test material only in the case of neutral ammonium citrate. Manganese oxide No. 2 added at a level of 10 ppm manganese was less available than manganese oxide No. 1 as measured by growth rate and leg development. However, manganese oxide No. 2 was more soluble in 0.4 percent hydrochloric acid and 2 percent citric acid than was manganese oxide No. 1. Thus, solubility of manganese sources in these solvents appears to be of limited usefulness in predicting availability of the element.

## Summary

Two experiments were conducted in an effort to develop a suitable biological assay for manganese availability with chicks. Reagent-grade manganese sulfate was used as the reference standard and was added to a semi-purified basal diet at levels of 10, 20 and 30 ppm manganese. Two commercial feed-grade manganese oxides were the other test materials, and were added at the levels of 10 and 20 ppm manganese to the same basal diet. Ten ppm supplemental manganese were adequate for normal growth and bone ash and to prevent the occurrence of perosis when supplied from the sulfate or one of the oxide sources. Ten ppm supplemental manganese from the other oxide source, however, resulted in reduced growth rates, a lower percent bone ash and a higher incidence of perosis.

Manganese concentration in the bone increased with increasing dietary levels of the element. This response suggests that the level of manganese

in the bone may be a sufficiently sensitive response criterion to be used in a manganese assay using manganese sulfate as the standard source.

## CHAPTER V

## EXPERIMENTS 4 AND 5. BIOLOGICAL AVAILABILITY OF SEVERAL INORGANIC FORMS OF MANGANESE TO CHICKS

Two experiments were conducted to determine the availability of manganese from several inorganic sources using the assay method suggested from the results of Experiments 2 and 3. An attempt was also made to demonstrate the relationship between the response criteria and levels of manganese intake above those tested in the first two experiments.

### Experimental Procedure

The basal diet used in the two identical experiments was the same as that used in Experiments 2 and 3 (Table 12). Upon analysis this diet was found to contain 5 ppm manganese. Treatment diets were prepared by adding the supplemental manganese source at the expense of glucose. The sources of manganese tested are shown in Table 18 along with the manganese content of each source, relative solubility in various solvents, and results of X-ray diffraction studies. Manganese content and solubility were determined as outlined in the experimental procedure for Experiments 2 and 3.

Each experiment consisted of 12 treatments and each treatment included two replicates of 10 chicks which were randomly assigned to experimental groups. The 12 treatments tested were basal diet, basal

TABLE 18. MANGANESE CONTENT, SOLUBILITY AND X-RAY PATTERNS OF MANGANESE SUPPLEMENTS

| Manganese Source | Manganese Content (%) | Solubility (%) | | | | Interpretation of X-ray Patterns |
|---|---|---|---|---|---|---|
| | | Water | 0.4% Hydrochloric Acid | 2% Citric Acid | Neutral Ammonium Citrate | |
| Standard | 31.16 | 100.0 | 100.0 | 100.0 | 100.0 | $MnSO_4 \cdot H_2O$ (Reagent Grade) |
| No. 2 | 24.00 | 0.3 | 91.0 | 58.0 | 2.7 | $MnCO_3$ (Rhodochrosite) Minor unidentified phases |
| No. 4 | 15.90 | 0.3 | 2.7 | 44.5 | 0.4 | Dolomite ($MgCaCO_3$), quartz ($SiO_2$), goethite ($HFeO_2$), calcite ($CaCO_3$) |
| No. 6 | 57.10 | 0.4 | 90.2 | 82.0 | 50.1 | Manganosite (MnO), $Mn_2 SiO_4$ Minor unidentified phases |
| No. 8 | 42.64 | 0.7 | 97.4 | 100.0 | 47.8 | $MnCO_3$ (Reagent Grade) |
| No. 12 | 57.29 | 0.7 | 13.9 | 56.6 | 9.8 | Bixbyite $(Fe, Mn)_2 O_3$, Hausmannite ($Mn_3O_4$), Manganosite (MnO), Pyrolusite ($MnO_2$) |
| No. 13 | 60.95 | 0.4 | 86.0 | 87.1 | 81.8 | Manganosite (MnO), Minor unidentified phases |

plus 10, 20, 30, 60 and 120 ppm manganese supplied from the reference standard, reagent-grade manganese sulfate, and basal plus 10 ppm supplemental manganese supplied from six other sources referred to as sources No. 2, 4, 6, 8, 12 and 13 as listed in Table 18. The manganese level of each diet was verified by chemical analysis.

As in Experiments 2 and 3, 240 day-old Leghorn cockerel chicks obtained from a local commercial hatchery were used in each experiment. Treatment groups were housed in a thermostatically controlled, electrically heated battery brooder constructed primarily of stainless steel with raised wire floors. Tap water (0.4 ppm manganese) and the experimental diets were supplied ad libitum to all chicks throughout the 28-day experimental period. At the end of the 28-day trial, individual body weights were recorded and all chicks were examined for abnormal leg development or perosis and scored according to the method outlined for Experiments 2 and 3. All chicks were killed after examination for perosis and the right tibiae and femurs removed for determination of bone ash and bone manganese levels as described for Experiments 2 and 3.

The data resulting from measurements of body weight, leg abnormalities, bone ash and bone manganese were subjected to analysis of variance and bone manganese data analyzed by regression and correlation analyses as outlined by Steel and Torrie (1960). Significant treatment differences were determined by Duncan's multiple range test (1955).

## Results

Statistical analyses of the data obtained from these experiments revealed a significant ($P < .01$) experiment x treatment interaction, therefore the data are presented separately.

The results of body weight determinations at 28 days are shown in Table 19 for both experiments. Those chicks receiving only the basal diet in Experiment 4 had an average body weight of 195 grams which was significantly ($P < .01$) lower than any other treatment group. There were no significant differences in body weights of 28-day-old chicks fed 10 ppm supplemental manganese either from the standard source or from sources No. 2, 4, 6 and 12 or 20 ppm from the standard source. There were no significant differences among groups fed 10 ppm manganese from sources No. 8 and No. 13 or 30 and 120 ppm from the standard source. Body weights were significantly ($P < .01$) greater for the chicks receiving 60 ppm supplemental manganese from the standard source (277 grams) than for those receiving the basal or basal plus 10 ppm manganese from the standard source or No.'s 2, 4, 6 and 12. Body weights were generally higher for 28-day-old chicks in Experiment 5. As in Experiment 4, feeding the basal with no supplemental manganese resulted in the lowest ($P < .01$) average value for 28-day body weight (227 grams). In contrast to the results of Experiment 4 however, there were no significant differences in body weights among the other treatment groups with an average weight of 271 grams recorded for these groups.

A summary of leg abnormality scores for both experiments is presented in Table 20. The group fed the basal diet in Experiment 4 had the highest incidence of leg abnormalities (2.78) but was not significantly different from groups receiving 10 ppm manganese from sources No. 2, 4, 6 and 12. There were no significant differences among the groups fed 10 ppm supplemental manganese from any source with an average value of 1.99 recorded for all of these treatments. Leg abnormalities were, for all practical purposes, absent in chicks fed more than 10 ppm supplemental manganese from the standard source in Experiment 4.

TABLE 19. BODY WEIGHTS OF 28-DAY-OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE[1]

| Manganese Source | Supplemental Manganese, ppm | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 60 | 120 |
| | Experiment 4 | | | | | |
| | (Body Wt., gm) | | | | | |
| Standard | 195[a] | 241[b,c] | 256[b,c,d] | 265[c,d] | 277[d] | 271[c,d] |
| No. 2 | --- | 240[b,c] | --- | --- | --- | --- |
| No. 4 | --- | 228[b] | --- | --- | --- | --- |
| No. 6 | --- | 226[b] | --- | --- | --- | --- |
| No. 8 | --- | 270[c,d] | --- | --- | --- | --- |
| No.12 | --- | 240[b,c] | --- | --- | --- | --- |
| No.13 | --- | 265[c,d] | --- | --- | --- | --- |
| | Experiment 5 | | | | | |
| | (Body Wt., gm) | | | | | |
| Standard | 227[a] | 280[b] | 289[b] | 265[b] | 264[b] | 275[b] |
| No. 2 | --- | 259[b] | --- | --- | --- | --- |
| No. 4 | --- | 271[b] | --- | --- | --- | --- |
| No. 6 | --- | 268[b] | --- | --- | --- | --- |
| No. 8 | --- | 283[b] | --- | --- | --- | --- |
| No.12 | --- | 259[b] | --- | --- | --- | --- |
| No.13 | --- | 266[b] | --- | --- | --- | --- |

[1] Values listed are means of 20 observations and different superscripts within experiment groups denote significant ($P < .01$) differences.

TABLE 20. LEG ABNORMALITY SCORES OF 28-DAY-OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE[1,2]

| Manganese Source | Supplemental Manganese, ppm | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 60 | ]20 |
| | Experiment 4 | | | | | |
| | (Leg Score, 0-4) | | | | | |
| Standard | 2.78[a] | 1.58[b] | 0.23[c] | 0.32[c] | 0.28[c] | 0.18[c] |
| No. 2 | --- | 2.48[a,b] | --- | --- | --- | --- |
| No. 4 | --- | 1.92[a,b] | --- | --- | --- | --- |
| No. 6 | --- | 2.25[a,b] | --- | --- | --- | --- |
| No. 8 | --- | 1.60[b] | --- | --- | --- | --- |
| No. 12 | --- | 2.30[a,b] | --- | --- | --- | --- |
| No. 13 | --- | 1.78[b] | --- | --- | --- | --- |
| | Experiment 5 | | | | | |
| | (Leg Score, 0-4) | | | | | |
| Standard | 3.20[a] | 0.35[e,f] | 0.08[f] | 0.01[f] | 0.09[f] | 0.22[f] |
| No. 2 | --- | 3.12[a] | --- | --- | --- | --- |
| No. 4 | --- | 1.64[b,c] | --- | --- | --- | --- |
| No. 6 | --- | 1.01[c,d,e] | --- | --- | --- | --- |
| No. 8 | --- | 0.43[d,e,f] | --- | --- | --- | --- |
| No. 12 | --- | 2.03[b] | --- | --- | --- | --- |
| No. 13 | --- | 1.18[c,d] | --- | --- | --- | --- |

[1] Values listed are means of 20 observations and different superscripts within experiment groups denote significant ($P < .01$) differences.

[2] Grading Scale: 0 = Normal leg
1 = Slight swelling of tibiometatarsal joint
2 = Marked swelling of joint
3 = Swelling and slipping of tendon
4 = Swelling and marked slipping of tendon

Feeding the basal diet or basal plus 10 ppm manganese from source No. 2 resulted in the highest ($P < .01$) incidence of leg abnormalities in Experiment 5. An average value of 2.03 was assigned to those chicks fed 10 ppm manganese from source No. 12. This was significantly ($P < .01$) greater than values assigned to those fed 10 ppm from the standard or from sources No. 6, 8 and 13 (0.74). However, this was not significantly different from the leg score for the group fed 10 ppm manganese from source No. 4. Again there was practically no occurrence of perosis in the groups fed more than 10 ppm supplemental manganese, and in this experiment these groups were not significantly different from those fed 10 ppm from the standard source or from source No. 8.

Figure 5 shows representative tibiae from the chicks receiving the basal diet and basal plus 10, 20, 30, 60 and 120 ppm manganese supplied from the standard source, manganese sulfate, in Experiment 5. The basal group had much shorter, thicker tibiae that were more bent at the ends than any of the groups fed supplemental manganese. The tibia length seemed to increase slightly with increasing levels of dietary manganese up to 20 ppm even though there were no differences in the assigned values for degree of leg abnormalities (Table 20) among the groups receiving varying levels of supplemental dietary manganese.

Representative tibiae from the groups fed 10 ppm manganese from the various test sources in Experiment 5 are shown in Figure 6. As was demonstrated by visual examination of the live bird (Table 20), 10 ppm manganese from sources No. 2, 4 and 12 resulted in shorter more extremely bent tibiae than did 10 ppm supplied from sources No. 8 and 13. However, the shorter tibiae for those chicks fed source No. 6 does not agree with the lower leg score assigned to live birds on this treatment.

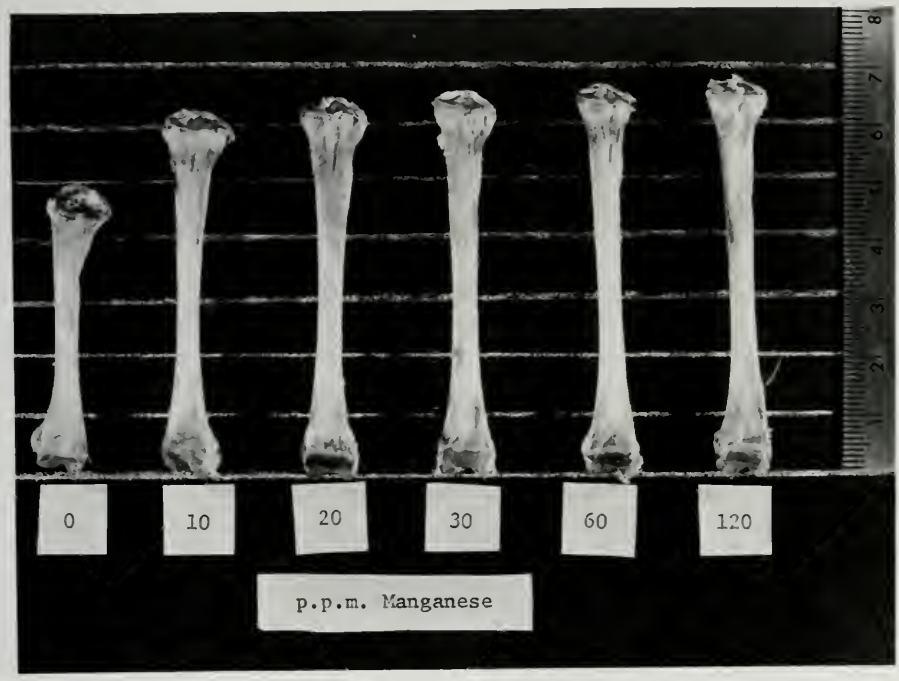


Figure 5. Representative Tibiae from 28-Day-Old Chicks Raised on Varying Levels of Dietary Manganese in Experiment 5.

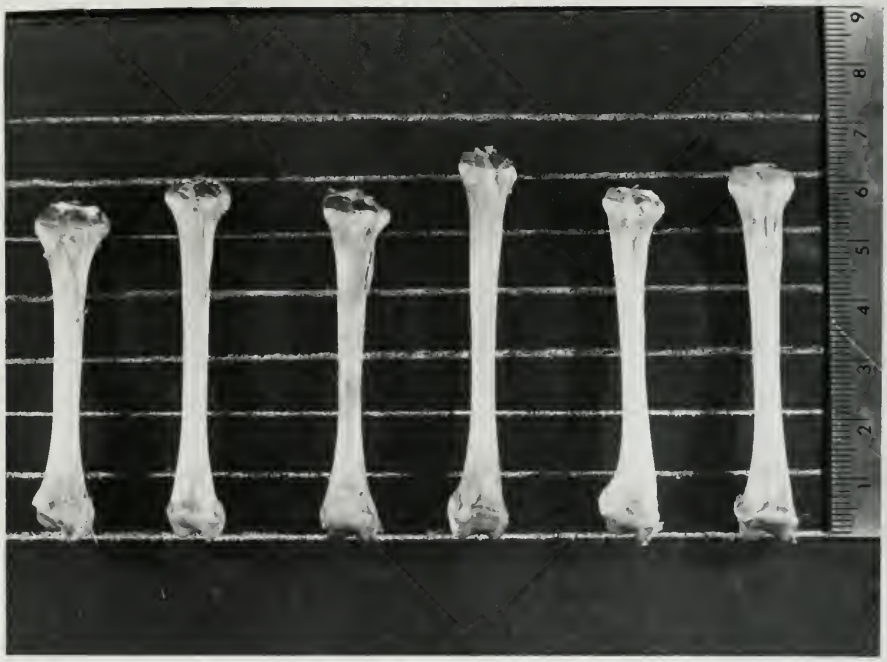

Figure 6. Representative Tibiae from 28-Day-Old Chicks Raised on a Diet Containing 10 ppm Supplemental Manganese Supplied from Various Sources in Experiment 5. Manganese Sources Represented from Left to Right are: No.'s 2, 4, 6, 8, 12 and 13.

Statistical analyses of the data obtained from bone ash determinations in Experiment 4 (Table 21) revealed no significant differences in percent bone ash among those groups fed the basal diet, any level of the standard source, or 10 ppm manganese from sources No. 8 and 12. These groups had an average of 46.9 percent bone ash. Those fed 10 ppm from source No. 6 (45.6 percent) had significantly ($P < .01$) less bone ash than the group fed source No. 4 (48.1 percent). Groups receiving sources No. 2 and 13 were intermediate in response.

Chicks fed the basal diet or basal plus 10 ppm supplemental manganese from the standard source or sources No. 2, 4, 8, 12 and 13 in Experiment 5 did not differ significantly in bone ash content (Table 21), averaging 46.2 percent. These groups were significantly lower in percentage of bone ash than the group fed 20 ppm manganese from the standard source (47.7 percent). Those receiving 10 ppm from source No. 6 or 30, 60 or 120 ppm of the element from the standard source had intermediate responses in bone ash levels.

Bone manganese data, expressed as ppm in dry, fat-free bone for these two experiments, are summarized in Table 22. Those chicks in Experiment 4 fed 10 ppm supplemental manganese supplied from source No. 2 had the lowest level of bone manganese. This level of 2.76 ppm was significantly ($P < .01$) less than the bone manganese concentrations of 3.56 and 3.47 ppm found for those receiving 10 ppm supplemental manganese from the standard source or source No. 13, respectively. There were no significant differences among the other treatment groups fed the basal diet or any level of the standard source less than 60 ppm. At 60 and 120 ppm however, bone manganese concentrations increased significantly ($P < .01$) with increases in dietary manganese.

TABLE 21. PERCENTAGE BONE ASH IN TIBIA AND FEMUR FROM 28-DAY-OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE[1]

| Manganese Source | Supplemental Manganese, ppm | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 60 | 120 |
| | Experiment 4 | | | | | |
| | (Percent in dry, fat-free bone) | | | | | |
| Standard | $46.8^{a,b,c}$ | $46.7^{a,b,c}$ | $46.9^{a,b,c}$ | $46.7^{a,b,c}$ | $47.3^{a,b,c}$ | $47.2^{a,b,c}$ |
| No. 2 | --- | $46.1^{a,b,c}$ | --- | --- | --- | --- |
| No. 4 | --- | $48.1^{c}$ | --- | --- | --- | --- |
| No. 6 | --- | $45.6^{a}$ | --- | --- | --- | --- |
| No. 8 | --- | $46.4^{a,b,c}$ | --- | --- | --- | --- |
| No. 12 | --- | $46.9^{a,b,c}$ | --- | --- | --- | --- |
| No. 13 | --- | $47.6^{b,c}$ | --- | --- | --- | --- |
| | Experiment 5 | | | | | |
| | (Percent in dry, fat-free bone) | | | | | |
| Standard | $46.0^{a}$ | $46.3^{a}$ | $47.7^{b}$ | $47.1^{a,b}$ | $47.0^{a,b}$ | $47.1^{a,b}$ |
| No. 2 | --- | $45.9^{a}$ | --- | --- | --- | --- |
| No. 4 | --- | $45.9^{a}$ | --- | --- | --- | --- |
| No. 6 | --- | $47.0^{a,b}$ | --- | --- | --- | --- |
| No. 8 | --- | $46.4^{a}$ | --- | --- | --- | --- |
| No. 12 | --- | $46.3^{a}$ | --- | --- | --- | --- |
| No. 13 | --- | $46.3^{a}$ | --- | --- | --- | --- |

[1] Values listed are means of 20 observations and different superscripts within experiment groups denote significant ($P < .01$) differences.

TABLE 22. BONE (TIBIA PLUS FEMUR) MANGANESE LEVELS OF 28-DAY-OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE[1]

| Manganese Source | Supplemental Manganese, ppm | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 60 | 120 |
| | Experiment 4 | | | | | |
| | (ppm in dry, fat-free bone) | | | | | |
| Standard | $3.27^{a,b,c}$ | $3.56^{c}$ | $3.53^{c}$ | $3.84^{c}$ | $5.24^{d}$ | $6.02^{e}$ |
| No. 2 | --- | $2.76^{a}$ | --- | --- | --- | --- |
| No. 4 | --- | $2.83^{a,b}$ | --- | --- | --- | --- |
| No. 6 | --- | $3.41^{a,b,c}$ | --- | --- | --- | --- |
| No. 8 | --- | $3.43^{a,b,c}$ | --- | --- | --- | --- |
| No. 12 | --- | $3.14^{a,b,c}$ | --- | --- | --- | --- |
| No. 13 | --- | $3.47^{b,c}$ | --- | --- | --- | --- |
| | Experiment 5 | | | | | |
| | (ppm in dry, fat-free bone) | | | | | |
| Standard | $2.90^{a,b}$ | $3.49^{b}$ | $4.31^{c}$ | $5.05^{d}$ | $5.82^{e}$ | $6.95^{f}$ |
| No. 2 | --- | $2.80^{a}$ | --- | --- | --- | --- |
| No. 4 | --- | $3.03^{a,b}$ | --- | --- | --- | --- |
| No. 6 | --- | $3.34^{a,b}$ | --- | --- | --- | --- |
| No. 8 | --- | $3.06^{a,b}$ | --- | --- | --- | --- |
| No. 12 | --- | $2.96^{a,b}$ | --- | --- | --- | --- |
| No. 13 | --- | $3.26^{a,b}$ | --- | --- | --- | --- |

[1] Values listed are means of 20 observations and different superscripts within experiment groups denote significant ($P < .01$) differences.

The concentration of manganese in dry, fat-free bone in Experiment 5 (Table 22) was, as in Experiment 4, significantly ($P < .01$) lower for the group fed 10 ppm supplemental manganese from source No. 2 (2.80 ppm) than in those chicks fed 10 ppm from the standard source (3.49 ppm). Groups fed the basal diet or basal plus 10 ppm added manganese from any of the other sources were intermediate in bone manganese levels and were not significantly different. As the level of supplemental manganese supplied from the standard source increased from 10 to 120 ppm, there was a corresponding significant ($P < .01$) rise in manganese levels of dry, fat-free bone.

Table 23 presents bone manganese levels for Experiments 4 and 5 expressed as ppm in bone ash. The level of manganese in bone ash for those chicks in Experiment 4 receiving 10 ppm manganese from source No. 4 was 5.91 ppm. This level was significantly lower than the level of 7.56 ppm recorded for those receiving 10 ppm from the standard source. There were no significant differences in manganese concentration in bone ash for the chicks fed the basal diet or basal plus 10 ppm of the element from any other source, with an average level of 6.98 ppm for these groups. Significant differences did not exist among the groups fed less than 60 ppm added manganese from the reference source. However, manganese levels in bone ash increased significantly ($P < .01$) to 11.10 and 12.90 ppm as supplemental levels increased to 60 and 120 ppm respectively.

Treatment responses for manganese concentration in bone ash in Experiment 5 (Table 23) were identical to the responses obtained when concentration was based on dry, fat-free bone (Table 22). The level of manganese was 6.10 ppm in bone ash for those chicks fed 10 ppm supplemental manganese from source No. 2. This was significantly ($P < .01$)

TABLE 23. BONE (TIBIA PLUS FEMUR) MANGANESE LEVEL OF 28-DAY-OLD CHICKS AS INFLUENCED BY SOURCE OR LEVEL OF SUPPLEMENTAL MANGANESE[1]

| Manganese Source | Supplemental Manganese, ppm | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 60 | 120 |
| | Experiment 4 (ppm in bone ash) | | | | | |
| Standard | $7.03^{a,b,c}$ | $7.56^{b,c}$ | $7.53^{b,c}$ | $8.23^{c}$ | $11.10^{d}$ | $12.90^{e}$ |
| No. 2 | --- | $6.00^{a,b}$ | --- | --- | --- | --- |
| No. 4 | --- | $5.91^{a}$ | --- | --- | --- | --- |
| No. 6 | --- | $7.46^{a,b,c}$ | --- | --- | --- | --- |
| No. 8 | --- | $7.40^{a,b,c}$ | --- | --- | --- | --- |
| No. 12 | --- | $6.69^{a,b,c}$ | --- | --- | --- | --- |
| No. 13 | --- | $7.32^{a,b,c}$ | --- | --- | --- | --- |
| | Experiment 5 (ppm in bone ash) | | | | | |
| Standard | $6.32^{a,b}$ | $7.56^{b}$ | $9.14^{c}$ | $10.75^{d}$ | $12.39^{e}$ | $14.79^{f}$ |
| No. 2 | --- | $6.10^{a}$ | --- | --- | --- | --- |
| No. 4 | --- | $6.58^{a,b}$ | --- | --- | --- | --- |
| No. 6 | --- | $7.10^{a,b}$ | --- | --- | --- | --- |
| No. 8 | --- | $6.59^{a,b}$ | --- | --- | --- | --- |
| No. 12 | --- | $6.39^{a,b}$ | --- | --- | --- | --- |
| No. 13 | --- | $7.05^{a,b}$ | --- | --- | --- | --- |

[1] Values listed are means of 20 observations and different superscripts within experiment groups denote significant ($P < .01$) differences.

lower than the level of 7.56 ppm found for those fed 10 ppm from the standard source. Again those fed the basal diet or 10 ppm added manganese from the other sources were not significantly different and had an average bone ash manganese concentration of 6.67 ppm. Bone manganese levels rose significantly ($P < .01$) from 7.56 ppm for those fed 10 ppm of the element from the standard source to 9.14, 10.75, 12.39 and 14.79 ppm as the supplemental manganese level increased from 10 to 20, 30, 60 and 120 ppm, respectively.

Significant ($P < .01$) correlations of 0.744 and 0.684 were found between the level of dietary manganese supplied from manganese sulfate and level of bone manganese (Figure 7) in Experiment 4. These correlation coefficients are for bone manganese expressed as ppm in dry, fat-free bone and ppm in bone ash respectively. In Experiment 4 the relationship between bone manganese levels and dietary manganese intake was found to be linear in nature. The linear regression equations obtained are as follows: $Y = 3.21 + 0.023X$ and $Y' = 6.93 + 0.048X$ where X represents the dietary manganese level, Y is the manganese level in dry, fat-free bone and Y' is the level of manganese in bone ash.

The relationships between dietary manganese intake and bone manganese levels in Experiment 5 are shown graphically in Figure 8. The "best-fitting" regression lines were found to be quadratic over the entire range of manganese intakes tested in this experiment (5-125 ppm). Statistical analyses of these data revealed significant ($P < .01$) correlations of 0.836 and 0.822 between manganese intake and manganese levels in dry, fat-free bone and bone ash respectively. Quadratic regression equations, $Y = 4.73 + 0.074\ (X-44.47) - 0.0003\ (X-44.47)^2$ and $Y' = 10.11 + 0.151\ (X-44.47) - 0.0006\ (X-44.47)^2$, were obtained where X

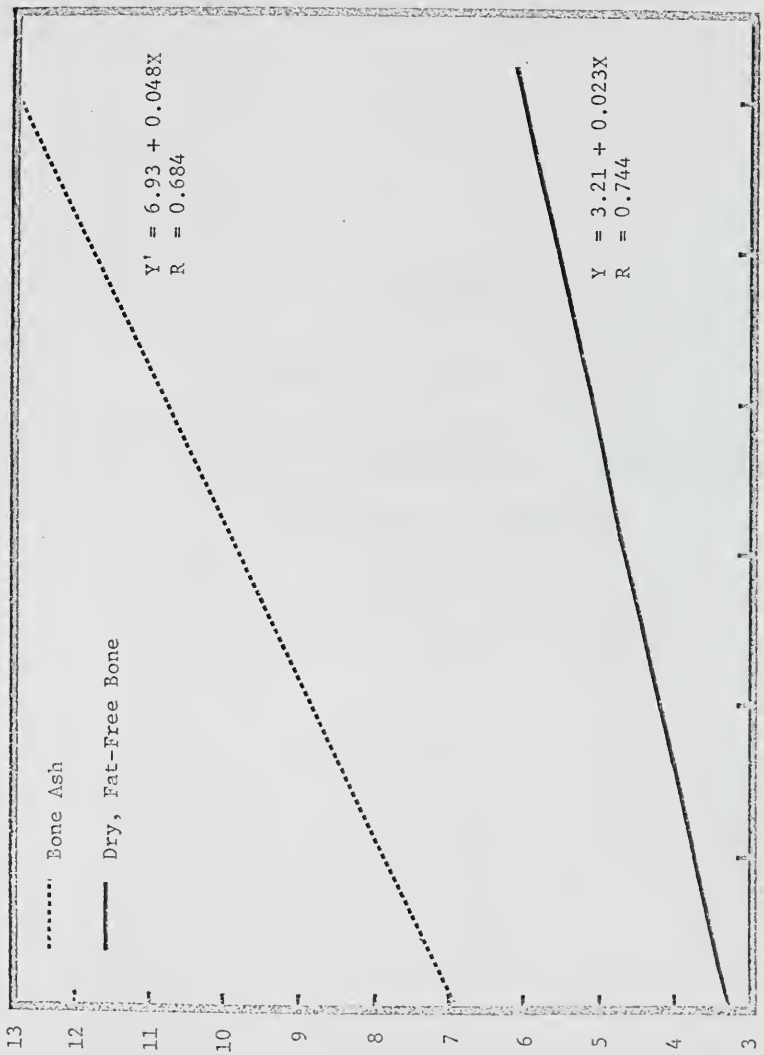


Figure 7. Relation of Bone (tibia plus femur) Manganese Levels to Manganese Intake in the Form of Manganese Sulfate (Experiment 4).

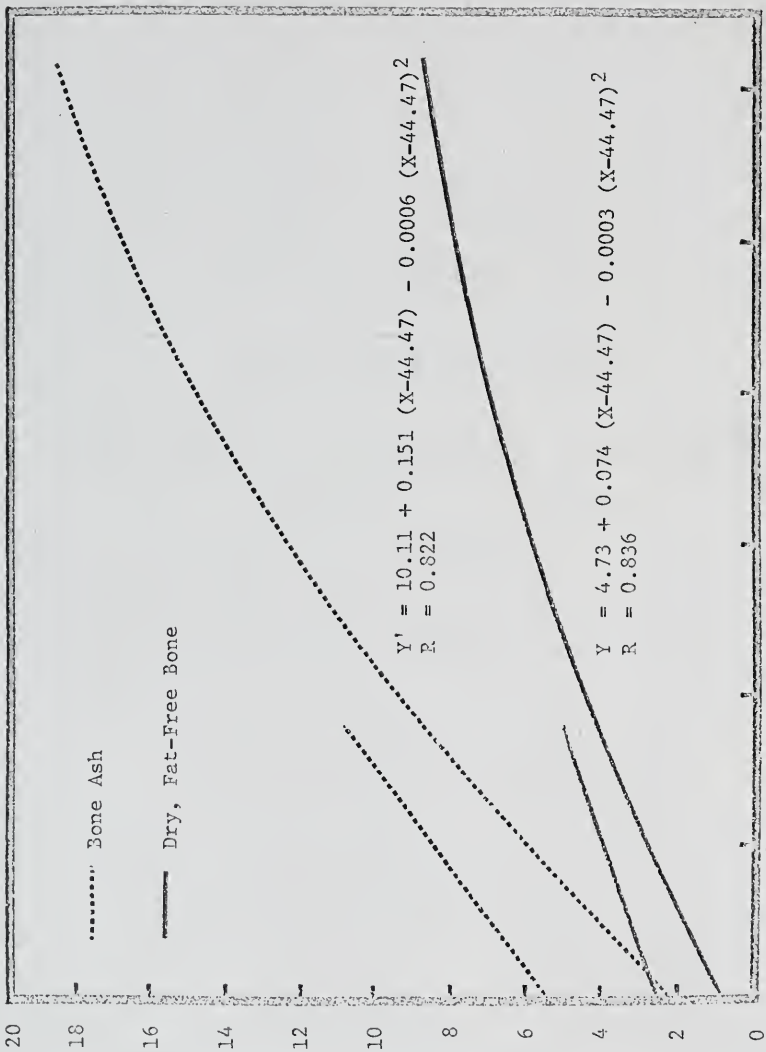
Bone Manganese, ppm
Dietary Manganese, ppm
Bone Ash
Dry, Fat-Free Bone
Y' = 10.11 + 0.151 (X-44.47) - 0.0006 (X-44.47)2
R = 0.822
Y = 4.73 + 0.074 (X-44.47) - 0.0003 (X-44.47)2
R = 0.836

Figure 8. Relation of Bone (tibia plus femur) Manganese Levels to Manganese Intake in the Form of Manganese Sulfate (Experiment 5).

is the manganese intake, Y is the concentration of manganese in dry, fat-free bone and Y' is the ppm of the element in bone ash.

Linear regression lines were the "best-fit" in Experiment 5 when bone manganese responses due to supplemental manganese at levels of 30 ppm and below were examined alone. The regression equations were $Y = 2.48 + 0.072X$ and $Y' = 5.47 + 0.149X$ where X is the manganese intake level, Y is the manganese level in dry, fat-free bone, and Y' is the level in bone ash.

## Discussion

Identical results in chick response were not obtained in Experiments 4 and 5. Variation within treatments was quite high in Experiment 4. Chicks in this experiment started growing at slow rate with much variation in growth within each pen of chicks. It was suspected that these chicks were "chilled" during transport from the hatchery to the experimental facilities.

The results of these experiments are in general agreement with the data obtained from Experiments 2 and 3. The basal diet, without supplemental manganese, resulted in less than maximum growth rate and was ineffective in preventing the occurrence of perosis. Growth rate was improved significantly by the addition of 10 ppm supplemental manganese from any of the test sources. However, supplemental manganese levels greater than 10 ppm from the standard source failed to improve growth rate further. Results of visual examination of chicks for perosis at 28-days of age and examination of tibiae removed from these chicks indicated that 20 ppm or more supplemental manganese prevented perosis. The test manganese sources differed in their effectiveness in preventing

the disorder when supplemented at the 10 ppm level indicating differences in biological availability. It appears that the standard source, source No. 8 and No. 13 were more available or at least were more effective than the other test sources in reducing the occurrence of perosis. Source No. 2 was the least effective in decreasing the number of leg abnormalities.

Conclusions concerning availability of manganese from the different sources cannot be drawn on the basis of bone ash response. In general, there were no differences in percent bone ash due to level or source of manganese.

Bone manganese levels reflected dietary levels of the element more directly than did growth rate or degree of leg abnormalities. This was especially true in Experiment 5 where responses were the same if bone manganese were expressed on the basis of either dry, fat-free bone or bone ash. Increases in dietary manganese in Experiment 5 resulted in significant increases in bone levels of the element in those chicks fed the standard source as was found in the previous experiments. As indicated by examination for perosis, source No. 2 was less available than the other test sources. This source generally resulted in bone manganese levels lower than those found when the standard source was fed at the same dietary level and not different from levels in the basal group.

The regression and correlation analyses for these experiments are also in agreement with the findings from the previous experiments. In Experiment 4 there was not a significant linear relationship between bone manganese and manganese intake at dietary levels below 35 ppm. However, in Experiment 5 as in the earlier work, treatment means fell on the linear regression line in almost every case when the effects of dietary manganese intake,

at 35 ppm and below, on bone manganese were examined. The quadratic relationship that was obtained as intakes of manganese increased in Experiment 5 was expected since this type of relationship is found with most biological systems.

These results indicated, as was suggested in Experiments 2 and 3, that bone manganese levels can be used as a criterion in a biological assay testing manganese availability. It is also concluded that tests for differences in availability should be conducted with levels of dietary manganese below 35 ppm since it is in this range that maximum response is obtained.

The relative availability of manganese from the various sources is shown in Table 24. The standard values, assumed to be 100, are those obtained from the regression analyses. Relative availability percentages were assigned at the 10 ppm level of supplemental manganese either including or excluding the effect of the basal diet which contained approximately 5 ppm manganese. In Experiment 5 the relationship between responses to sources was the same whether measured on the basis of manganese concentration in dry, fat-free bone or in bone ash. There were slight differences in the relationship between sources in Experiment 4 when different response criteria were used. The general relationships, however, were the same in all cases. Sources No. 2, 4 and 12 were the least available in every case. Availabilities ranged from approximately 77 to 97 percent when the effect of the basal diet was included and from 0 to 68 percent when the effect of the basal diet was eliminated.

Solubilities of test materials were in better agreement with availabilities of these materials in the present experiments than in Experiments 2 and 3. However, as was found in the previous experiments,

TABLE 24. RELATIVE AVAILABILITY OF MANGANESE TEST SOURCES AT A SUPPLEMENTAL LEVEL OF 10 PPM[1]

| Manganese Source | Response Criteria: Mn Level in Dry, Fat-free Bone — Supplemental Plus Basal | Mn Level in Dry, Fat-free Bone — Supplemental Minus Basal | Mn Level in Bone Ash — Supplemental Plus Basal | Mn Level in Bone Ash — Supplemental Minus Basal |
|---|---|---|---|---|
| | Experiment 4 | | | |
| Standard[2] | 100.00 | 100.00 | 100.00 | 100.00 |
| No. 2 | 77.48 | 0 | 78.42 | 0 |
| No. 4 | 79.45 | 0 | 77.24 | 0 |
| No. 6 | 95.73 | 34.49 | 97.50 | 60.25 |
| No. 8 | 96.29 | 43.12 | 96.72 | 47.75 |
| No. 12 | 88.15 | 0 | 87.44 | 0 |
| No. 13 | 97.41 | 60.37 | 95.67 | 31.08 |
| | Experiment 5 | | | |
| Standard[2] | 100.00 | 100.00 | 100.00 | 100.00 |
| No. 2 | 78.34 | 0 | 79.24 | 0 |
| No. 4 | 84.78 | 25.18 | 85.47 | 24.70 |
| No. 6 | 93.45 | 67.82 | 92.23 | 59.72 |
| No. 8 | 85.62 | 29.31 | 85.60 | 25.38 |
| No. 12 | 82.82 | 15.55 | 83.00 | 11.91 |
| No. 13 | 91.21 | 56.82 | 91.58 | 56.35 |

[1] Availability values of zero indicate that response was less than that of the basal group.

[2] Values shown for test sources are percentages relative to the standard response.

solubility and availability were directly related only in the case of solubility in neutral ammonium citrate. Sources No. 2, 4 and 12 which were the least available test materials used in these experiments also exhibited the lowest solubilities in this solvent. All of the test sources were essentially insoluble in water.

## Summary

Two identical experiments were conducted to determine the availability of manganese from several inorganic sources of the element. The response criteria used in the assay were body weight, incidence of leg abnormalities, percent bone ash, and bone levels of manganese. Reagent-grade manganese sulfate, used as the reference standard, was added to the basal semi-purified diet at supplemental manganese levels of 10, 20, 30, 60 and 120 ppm. Six other inorganic sources of manganese were tested at a level of 10 ppm added manganese.

A level of 10 ppm supplemental manganese from any of the sources used was adequate to promote normal growth and bone ash levels. It was concluded that 20 ppm supplemental manganese from the standard source was required to prevent perosis entirely. The test sources differed in their effectiveness in preventing leg abnormalities at the 10 ppm supplemental manganese level.

Bone manganese level, when expressed as ppm in dry, fat-free bone or in bone ash, increased significantly with increasing levels of dietary manganese. Manganese concentration in the bone increased in a linear fashion at least up to 30 ppm added dietary manganese. Thus it is concluded that tests for biological availability should be conducted at supplemental levels below 30 ppm in the area of maximum response.

Differences in availability of manganese from the test sources were found when bone manganese levels were expressed as percentages relative to the response obtained with the standard, manganese sulfate, when supplied at the same supplemental level.

# CHAPTER VI

## SUMMARY AND CONCLUSIONS

Experiments were conducted with sheep and poultry to gain information on certain nutritional aspects of dietary manganese. The aspects studied involved the influence of dietary manganese level on absorption and utilization of manganese and other dietary minerals by sheep, and the development of a suitable biological assay for manganese availability with evaluation of the biological availability of several inorganic sources of this element for chicks. A summary of the results of these studies is presented in the following statements.

### Absorption, Blood Clearance, Tissue Deposition and Excretion of Manganese and Other Elements in Sheep

The values obtained for apparent absorption and net retention of stable manganese indicate that manganese is absorbed from the intestinal tract to a very limited extent. Absorption and retention were not significantly affected by dietary manganese level with excretion of manganese being approximately equal to dietary intake of the element. Limited absorption of manganese was also demonstrated by the extremely low plasma and tissue levels of $^{54}Mn$ in those lambs given an oral dose of the radioisotope. Plasma manganese levels remain quite low, appearing to be regulated by some type of homeostatic control mechanism. This mechanism

of control may be partially responsible for the low absorption of manganese from the intestinal tract. Even though significantly affected by dietary manganese levels, plasma levels of stable manganese remained within a narrow range. This rigid control of blood manganese levels is also demonstrated by the extremely rapid plasma clearance of intravenous $^{54}Mn$ injections.

The experimental results demonstrate that manganese stores within the body can be increased by increasing dietary intake of the element. Those lambs receiving the basal diet plus 4,000 ppm added manganese had significantly greater levels of manganese in all tissues analyzed than those fed only the basal diet. Also, deposition of $^{54}Mn$ in the tissues was higher for those animals fed the unsupplemented diet. The tissues, listed in decreasing order of enrichment with radioactive manganese, were as follows: kidney, liver, spleen, brain, heart, bone and muscle.

Excretion of stable manganese from the body was almost exclusively by way of the feces. Even though urinary manganese increased with increasing dietary manganese it still accounted for less than 1 percent of the total manganese excretion. Likewise, fecal $^{54}Mn$ accounted for essentially all excretion of the radioisotope in animals given either an intravenous or oral dose of the nuclide.

This research demonstrated interrelationships between manganese and other minerals. The high manganese diet resulted in a decreased intestinal absorption of iron and phosphorus and decreased liver concentrations of iron and zinc. Dietary manganese level did not significantly affect apparent absorption of copper, magnesium, calcium and zinc or tissue levels of phosphorus, copper, magnesium and calcium.

Biological Assay for Manganese Availability in the Chick

Body weight and percentage bone ash were not sensitive enough as response criteria to detect differences in biological availability among inorganic sources of manganese. The only consistent significant differences in body weight were between the groups fed only the basal semi-purified diet (4-5 ppm manganese) and those receiving 10 ppm supplemental manganese from manganese sulfate, the standard source. Bone ash was increased by the addition of supplemental manganese only in isolated instances in the four experiments conducted.

The manganese sources tested differed in their effectiveness in preventing perosis or leg abnormalities at the 10 ppm supplemental manganese level. Perosis was prevented entirely, however, when 20 ppm manganese or greater were added to the diet used in these experiments. Thus, it appears that incidence of leg abnormalities is a useful response criterion for manganese availability when comparisons are made at dietary manganese levels of 10 to 15 ppm.

Bone concentrations of manganese were more directly related to dietary levels of the element over the entire supplemental range tested than were the other response criteria. The results of this research indicate that bone manganese concentrations increase in a linear manner as dietary levels increase up to 35 ppm. Although bone manganese continued to increase with increases in dietary manganese, the response was not always linear above 35 ppm manganese. Thus, it is concluded that tests for manganese availability should be conducted at dietary levels below 35 ppm where the maximum response is attained. Differences in availability of manganese from the test sources used in these trials were found when bone manganese levels were expressed as percentages relative

to the response obtained with the standard, manganese sulfate, when supplied at the same supplemental manganese level.

It is concluded from these data that meaningful values for manganese availability from different sources can be obtained using manganese sulfate as the reference standard and bone manganese concentration and incidence of perosis as the main response criteria. It also appears that tests for availability should be conducted at total dietary manganese levels of about 10 to 15 ppm.

# APPENDIX

TABLE 25. LIVER LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGNAESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$

| Dietary Mn Level | Isotope Admin. Pathway | Element | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Ca | Mg | P |
| | | (Concentration, ppm) | | | | | | |
| Low | IV | 8.4 | 1150 | 69.5 | 204 | 171 | 595 | 12,069 |
| | | 8.1 | 1120 | 36.5 | 211 | 156 | 565 | 10,645 |
| | | 10.9 | 441 | 51.7 | 200 | 140 | 585 | 11,395 |
| | | 11.5 | 1374 | 41.0 | 229 | 161 | 607 | 10,332 |
| Low | Oral | 13.8 | 1239 | 32.8 | 161 | 166 | 581 | 12,844 |
| | | 6.9 | 1289 | 33.7 | 185 | 167 | 567 | 13,589 |
| | | 8.7 | 1097 | 40.7 | 181 | 184 | 589 | 11,300 |
| | | 10.5 | 733 | 36.3 | 176 | 156 | 612 | 11,566 |
| High | IV | 50.2 | 746 | 67.6 | 174 | 152 | 625 | 12,324 |
| | | 35.2 | 880 | 45.7 | 207 | 178 | 634 | 12,134 |
| | | 40.6 | 487 | 62.0 | 171 | 168 | 654 | 12,109 |
| | | 41.9 | 750 | 68.5 | 148 | 146 | 579 | 12,807 |
| High | Oral | 47.2 | 635 | 78.3 | 165 | 133 | 575 | 11,413 |
| | | 37.5 | 608 | 49.2 | 180 | 194 | 642 | 12,112 |
| | | 41.2 | 823 | 62.1 | 139 | 150 | 574 | 11,106 |
| | | 59.7 | 657 | 64.2 | 147 | 139 | 577 | 12,466 |

TABLE 26. KIDNEY LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$

| Dietary Mn Level | Isotope Admin. Pathway | Element | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Ca | Mg | P |
| | | (Concentration, ppm) | | | | | | |
| Low | IV | 2.8 | 302 | 15.0 | 82 | 364 | 574 | ------- |
| | | 4.2 | 300 | 16.6 | 165 | 389 | 754 | 10,454 |
| | | 6.3 | 227 | 22.9 | 146 | 380 | 869 | 10,928 |
| | | 4.0 | 278 | 20.1 | 201 | 516 | 745 | 10,698 |
| Low | Oral | 5.1 | 210 | 12.1 | 98 | 497 | 586 | 10,485 |
| | | 6.0 | 270 | 18.1 | 130 | 360 | 711 | 12,250 |
| | | 6.1 | 237 | 17.1 | 146 | 332 | 803 | 12,078 |
| | | 6.9 | 318 | 17.7 | 158 | 356 | 869 | 13,922 |
| High | IV | 20.0 | 194 | 22.1 | 182 | 326 | 715 | 11,776 |
| | | 15.3 | 379 | 22.5 | 147 | 368 | 799 | 12,267 |
| | | 24.4 | 171 | 18.5 | 166 | 540 | 844 | 12,717 |
| | | 27.1 | 229 | 18.4 | 182 | 385 | 798 | 12,890 |
| High | Oral | 22.4 | 160 | 19.3 | 154 | 383 | 785 | 11,166 |
| | | 19.8 | 210 | 17.0 | 151 | 532 | 787 | 10,742 |
| | | 23.8 | 274 | 16.8 | 180 | 353 | 708 | 11,295 |
| | | 26.7 | 178 | 18.3 | 161 | 338 | 804 | 11,848 |

TABLE 27. HEART LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$

| Dietary Mn Level | Isotope Admin. Pathway | Element | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Ca | Mg | P |
| | | (Concentration, ppm) | | | | | | |
| Low | IV | 2.14 | 274 | 14.7 | 76.0 | 197 | 825 | 9,114 |
| | | 2.43 | 285 | 17.7 | 89.3 | 204 | 889 | 9,566 |
| | | 2.37 | 259 | 19.5 | 89.6 | 215 | 889 | 9,464 |
| | | 2.34 | 266 | 14.7 | 88.0 | 192 | 938 | 9,612 |
| Low | Oral | 2.90 | 327 | 15.0 | 83.0 | 174 | 970 | 9,734 |
| | | 2.62 | 280 | 16.5 | 89.3 | 186 | 891 | 9,827 |
| | | 2.08 | 258 | 17.8 | 86.5 | 172 | 957 | 10,399 |
| | | 2.54 | 310 | 19.0 | 81.4 | 179 | 801 | 9,750 |
| High | IV | 5.23 | 284 | 16.3 | 77.7 | 189 | 875 | 9,050 |
| | | 4.51 | 299 | 19.8 | 89.6 | 215 | 898 | 10,334 |
| | | 5.40 | 278 | 16.0 | 89.6 | 174 | 847 | 9,694 |
| | | 5.65 | 261 | 15.9 | 84.4 | 188 | 837 | 9,677 |
| High | Oral | 5.04 | 257 | 16.3 | 82.0 | 201 | 974 | 9,802 |
| | | 4.72 | 249 | 17.6 | 88.8 | 201 | 877 | 9,490 |
| | | 5.43 | 287 | 19.5 | 87.7 | 219 | 910 | 11,582 |
| | | 6.77 | 262 | 19.5 | 88.2 | 199 | 964 | 10,628 |

TABLE 28. SPLEEN LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$

| Dietary Mn Level | Isotope Admin. Pathway | Element | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Ca | Mg | P |
| | | (Concentration, ppm) | | | | | | |
| Low | IV | 1.57 | 3433 | 6.9 | 143 | 220 | 712 | 12,384 |
| | | 1.94 | 4024 | 15.7 | 153 | 259 | 836 | 14,251 |
| | | 2.18 | 4112 | 5.1 | 121 | 221 | 728 | 12,999 |
| | | 2.13 | 5382 | 8.0 | 139 | 263 | 773 | 11,514 |
| Low | Oral | 6.13 | 6186 | 19.6 | 172 | 288 | 936 | 11,429 |
| | | 2.14 | 4379 | 6.6 | 141 | 182 | 615 | 11,975 |
| | | 2.39 | ---- | 6.8 | 120 | 187 | 635 | 12,169 |
| | | 1.91 | 3258 | 5.6 | 141 | 183 | 782 | 14,883 |
| High | IV | 5.94 | 2785 | 6.1 | 113 | 209 | 609 | ------ |
| | | 5.05 | 2880 | 5.7 | 121 | 226 | 714 | 13,336 |
| | | 4.58 | 2060 | 7.4 | 140 | 176 | 784 | 14,766 |
| | | 5.54 | 1500 | 9.8 | 135 | 212 | 724 | 14,136 |
| High | Oral | 10.83 | 7483 | 8.8 | 172 | 203 | 725 | 9,968 |
| | | 6.07 | 3277 | 12.3 | 146 | 255 | 751 | 12,395 |
| | | 8.14 | 1456 | 8.8 | 123 | 219 | 701 | 12,832 |
| | | 4.86 | 3012 | 7.2 | 120 | 193 | 730 | 12,372 |

TABLE 29. MUSCLE LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$

| Dietary Mn Level | Isotope Admin. Pathway | Elements | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Ca | Mg | P |
| | | (Concentration, ppm) | | | | | | |
| Low | IV | 0.97 | 137 | 8.7 | 90 | 94 | 834 | 7,649 |
| | | 1.00 | 146 | 6.1 | 128 | 132 | 810 | 6,926 |
| | | 1.07 | 121 | 7.1 | 84 | 116 | 776 | 6,690 |
| | | 1.26 | 151 | 5.9 | 169 | 161 | 935 | 8,127 |
| Low | Oral | 1.03 | 127 | 7.4 | 137 | 130 | 823 | 7,591 |
| | | 1.38 | 131 | 6.3 | 132 | 133 | 888 | 8,615 |
| | | 0.60 | 112 | 4.4 | 114 | 108 | 640 | 6,404 |
| | | 0.75 | 114 | 1.7 | 150 | 113 | 711 | 6,758 |
| High | IV | 2.10 | 123 | 4.1 | 152 | 161 | 897 | 8,422 |
| | | 1.03 | 106 | 4.0 | 112 | 136 | 950 | 8,507 |
| | | 0.88 | 90 | 3.6 | 141 | 112 | 823 | 7,650 |
| | | 1.23 | 94 | 3.2 | 110 | 113 | 749 | 7,252 |
| High | Oral | 2.25 | 175 | 10.4 | 101 | 130 | 871 | 8,242 |
| | | 1.62 | 114 | 5.0 | 129 | 139 | 808 | 8,527 |
| | | 2.28 | 155 | 4.1 | 106 | 123 | 870 | 8,501 |
| | | 1.25 | 106 | 4.9 | 109 | 103 | 769 | 6,663 |

TABLE 30. BONE LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$

| Dietary Mn Level | Isotope Admin. Pathway | Element | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Ca | Mg | P |
| | | (Concentration, ppm) | | | | | | |
| Low | IV | 4.6 | 27.3 | 2.65 | 345 | 311,949 | 15,735 | 169,856 |
| | | 5.2 | 37.2 | 2.67 | 366 | 321,047 | 17,811 | 183,152 |
| | | 5.2 | 29.5 | 1.86 | 315 | 314,710 | 15,785 | 179,975 |
| | | 5.2 | 37.6 | 2.26 | 351 | 312,720 | 15,959 | 179,305 |
| Low | Oral | 5.7 | 44.4 | 2.51 | 288 | 308,272 | 14,413 | 172,893 |
| | | 5.4 | 41.1 | 3.14 | 305 | 308,390 | 14,637 | 187,480 |
| | | 5.4 | 26.1 | 3.86 | 293 | 318,550 | 14,468 | 193,530 |
| | | 5.0 | 26.2 | 3.30 | 301 | 301,790 | 15,065 | 180,179 |
| High | IV | 12.4 | 30.2 | 4.28 | 280 | 322,398 | 16,412 | 168,254 |
| | | 10.1 | 37.8 | 4.00 | 304 | 315,765 | 15,501 | 177,007 |
| | | 17.6 | 26.7 | 2.99 | 295 | 313,138 | 16,447 | 173,491 |
| | | 20.1 | 30.6 | 3.76 | 301 | 329,545 | 16,740 | 173,893 |
| High | Oral | 9.8 | 28.2 | 4.13 | 344 | 321,886 | 13,106 | 180,077 |
| | | 11.9 | 36.6 | 4.80 | 362 | 321,324 | 13,932 | 178,512 |
| | | 15.9 | 41.1 | 5.19 | 296 | 311,827 | 12,732 | 174,765 |
| | | 16.1 | 41.1 | 3.78 | 414 | 315,049 | 13,688 | 177,166 |

TABLE 31. BRAIN LEVELS OF VARIOUS ELEMENTS IN LAMBS FED DIFFERENT LEVELS OF DIETARY MANGANESE AND DOSED EITHER ORALLY OR INTRAVENOUSLY WITH $^{54}Mn$

| Dietary Mn Level | Isotope Admin. Pathway | Element | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Mn | Fe | Cu | Zn | Ca | Mg | P |
| | | (Concentration, ppm) | | | | | | |
| Low | IV | ---- | --- | ---- | ---- | --- | --- | ------ |
| | | ---- | --- | ---- | ---- | --- | --- | ------ |
| | | 2.5 | 173 | 13.7 | 69.1 | 302 | 665 | 15,596 |
| | | 2.7 | 197 | 14.4 | 77.5 | 314 | 680 | 16,308 |
| Low | Oral | ---- | --- | ---- | ---- | --- | --- | ------ |
| | | ---- | --- | ---- | ---- | --- | --- | ------ |
| | | 4.8 | 213 | 16.2 | 75.4 | 308 | 635 | 15,525 |
| | | 2.4 | 157 | 13.6 | 68.5 | 318 | 638 | 15,456 |
| High | IV | 12.1 | 162 | 13.1 | 68.0 | 345 | 622 | 14,573 |
| | | 10.7 | 135 | 14.9 | 76.7 | 263 | 643 | 16,113 |
| | | 17.4 | 170 | 16.1 | 71.9 | 276 | 659 | 15,177 |
| | | 18.0 | 169 | 15.6 | 65.4 | 336 | 645 | 14,816 |
| High | Oral | 15.0 | 154 | 15.1 | 63.8 | 306 | 647 | 16,198 |
| | | 13.7 | 139 | 18.5 | 69.2 | 301 | 608 | 14,689 |
| | | 15.5 | 127 | 15.6 | 65.4 | 307 | 609 | 15,493 |
| | | 14.4 | 204 | 14.6 | 66.9 | 304 | 622 | 15,179 |

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He is a member of Phi Kappa Phi, Gamma Sigma Delta, Sigma Xi, Alpha Zeta, Phi Sigma Society and the American Society of Animal Science.

He is married to the former Miss Sherrol Elaine Lynn and has one son, David Thomas.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

C. B. Ammerman, Chairman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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December, 1970

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